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UDIES ON BACTERIUM COLI CLOSELY RELATED FORMS

UC-NRLF
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BY

MAX LEVINE

THESIS

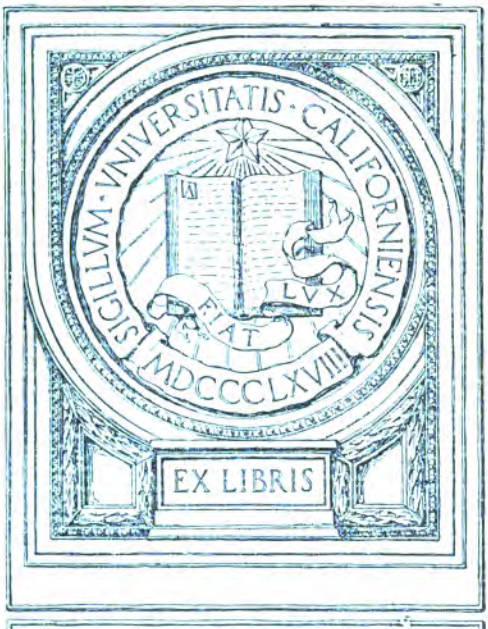
Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY IN BACTERIOLOGY

In

THE GRADUATE SCHOOL
of the
UNIVERSITY OF IOWA
1922

EXCHANGE



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NOTES ON BACT. COLI AND BACT. AEROGENES

MAX LEVINE,
From the Department of Pathology and Bacteriology, State University of Iowa, Iowa City, Iowa.

Read before Laboratory Section, American Public Health Association, at San Francisco, Cal., September 16, 1920.

Accurate information on the relative incidence of Bact. coli and Bact. aërogenes in nature would aid materially in the interpretation of the colon test in water analysis. Professor Levine suggests the lines along which selective media, for the isolation of these organisms, may be devised.

IN studies on the distribution of *B. coli* and *B. aërogenes,* the author has given preference to the plate method of isolation. This, also, seems to be the view of most other investigators who have concerned themselves with similar studies. In water analysis, on the other hand, the plate method of direct isolation is inconvenient and practically impossible when dealing with large samples (10 to 100 cc.), and preliminary enrichment, therefore, is resorted to. What happens in the preliminary enrichment tube as to the relative abundance of *B. coli* and *B. aërogenes,* is not definitely known as far as the author is aware. Overgrowths of one or the other of these organisms in the preliminary enrichment tube make it extremely difficult, if not impossible, to correlate the water results with the findings that have been reported (with the plate method) on the distribution of *B. coli* and *B. aërogenes* in nature.

The author has felt for some time that before much light will be thrown on the true relative incidence of *B. coli* and *B. aërogenes* in water and in feces, etc., it will be necessary first, so to modify our preliminary enrichment media, or other conditions, as to enable the investigator to isolate or suppress either *B. coli* or *B. aërogenes* at will.

With this in mind, studies were begun to determine the influence of various factors such as dyes, bile-salts, concentration of peptone, etc., on the rate of multiplication of *B. coli.* In general, it was found—

(1) That *B. coli* would not grow in ½ percent peptone with crystal violent in a dilution of 1-200,000, or brilliant green in a dilution of 1-1,000,000.

(2) That bile-salts stimulated the growth of *B. coli* when the concentration was less than 0.5 percent, but showed a marked inhibitory action if the concentration were raised to 0.7 or 1.0 percent. It was intended to continue this work with *B. aërogenes,* but the outbreak of the war interfered with the plans. The following factors are now being studied as to their influence on the growth of *B. coli* and *B. aërogenes.*

1. Temperature.
2. Boric acid.
3. Crystal violet.
4. Brilliant green.

Temperature.—That *B. coli* and *B. aërogenes* have different optimum growth temperatures may be inferred from the literature. Rogers and his associates have often mentioned the necessity for using a relatively low temperature (30° C.) for growth of some strains of *B. aërogenes* isolated from grains. Similarly Rettger reports that in studying the distribution of the colon group in unpolluted soils a temperature of 30° C.

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was desirable for isolation of the *B. aërogenes* types.

As to *B. coli*, a temperature of 40° C. has often been recommended for its isolation and in the Eijkman test 46° C. is employed for the isolation of the organism from water. In fact, it has been observed that the maximum rate of multiplication of *B. coli* is at about 45° C.

The author observed that in peptone lactose media at 43° C. (in a water bath) all the cultures of *B. coli* (16) grew luxuriantly as evidenced by strong turbidity in 24 hours, but 69 percent showed no gas or only a bubble in 24 hours. Of 20 cultures of *B. aërogenes*, on the other hand, 16 showed no growth, 2 slight, and 2 grew luxuriantly.

Boric Acid.—In agar of the following composition: peptone 1.0 percent, agar 1.5 percent, dipotassium phosphate 0.3 percent, and glucose .05 percent with 0.63 percent of boric acid, *B. aërogenes* failed to grow, whereas *B. coli* grew luxuriantly. In liquid media, 1.0 percent peptone with 0.63 percent boric acid, *B. coli* multiplied slowly, while *B. aërogenes* died off as evidenced by the following figures:

Culture 19b, *B. coli* increased from 65,000 per c.c. to 1,500,000 per c.c. in 48 hours, while *B. aërogenes* was reduced from 2,300 per cc. to 20 in 24 hours and to 0 in 48 hours. It was found in subsequent studies, however, that the difference in concentration of boric acid, which did not inhibit *B. coli* and which did inhibit *B. aërogenes*, was so close that it could not be safely employed as a selective agent.

Crystal Violet.—One percent peptone water containing ½ percent lactose and varying concentrations of crystal violet were inoculated from 48-hour peptone cultures of *B. coli* and *B. aërogenes*. Five different strains of each species were employed. A concentration of 1-100,000 of crystal violet prevented the growth of all the cultures of *B. coli*, whereas all of the *B. aërogenes* grew heavily. One culture of *B. coli* failed to grow in a dilution of 1-250,000 of crystal violet.

Decreasing the concentration of peptone to ½ percent increased markedly the inhibitory action of the dye. Thus in ½ percent peptone lactose solution none of the *B. aërogenes* grew with a dye concentration of 1-100,000, but all grew luxuriantly in 1-250,000 crystal violet. Among the *B. coli* cultures, all were inhibited in 1-250,000 dilution of the dye and two failed to grow in a dilution of 1-500,000.

Brilliant Green.—Some time ago, the author was informed that growths of *B. coli* are rarely encountered in the isolation of *B. typhosus* from stools by the use of eosine brilliant green agar, and that if a growth other than *B. typhosus* was present, it was very likely to be *B. aërogenes*. This suggested that the inhibitory action of brilliant green was much greater for *B. coli* than for *B. aërogenes*.

In a medium consisting of 1.0 percent peptone and 0.5 percent lactose with various concentrations of brilliant green, four cultures of *B. aërogenes* grew very luxuriantly in a concentration of 1-750,000, whereas one failed to grow in this concentration, but grew very well in 1-1,000,000 dilution of the dye. The 5 cultures of *B. coli*, on the other hand, all failed to grow in 1-750,000 of the dye, 3 did not grow in a dilution of 1-1,000,000 and 2 failed to grow even in a dilution of 1-1,500,000.

Reducing the concentration of peptone to ½ percent increased very markedly the antiseptic action of brilliant green. The five *B. coli* cultures now failed to grow even in a dilution of 1-3,000,000. The *B. aërogenes* cultures grew luxuriantly in a dilution of 1-2,000,000. Four grew in a dilution of 1-1,500,000, but only 1 grew in more concentrated solutions of the dye.

The selective action of brilliant green was even more strikingly shown by the use of a plate medium consisting of the simplified eosine methylene blue agar

with various concentrations of the brilliant green. Four cultures of *B. aërogenes* and five of *B. coli* were employed with the following results:

With a dilution of 1-100,000 of brilliant green, none of the *B. coli* grew at all. All of the *B. aërogenes* grew, but the colonies were only half as large as the controls indicating a marked inhibition. With 1-200,000 dilution, *B. coli* still failed to grow whereas *B. aërogenes* grew reasonably well, but not as luxuriantly as the controls. With 1-300,000 of the dye, three of the *B. coli* still failed to show any evidence of growth and two others grew very poorly. All the aërogenes showed a very heavy growth. With 1-400,000 brilliant green, the growths of *B. aërogenes* were as luxuriant as the controls, whereas 2 cultures of *B. coli* failed to grow and the three others showed very small, stunted non-characteristic colonies.

In conclusion, it may be said that these preliminary studies indicate that the concentration of peptone exerts a marked influence on the inhibitory action of dyes in culture media, and that it appears feasible to devise both liquid and solid media which will inhibit *B. coli,* but not *B. aërogenes.* The most promising inhibitory agent which we have as yet encountered for this purpose is brilliant green.

Acid-Production and Other Characters of Bacillus-Coli-Like Bacteria from Feces and Sewage

MAX LEVINE

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ACID-PRODUCTION AND OTHER CHARACTERS OF BACILLUS-COLI-LIKE BACTERIA FROM FECES AND SEWAGE [1]

MAX LEVINE

From the Department of Bacteriology of the Iowa State College, Ames

The ability to decompose carbohydrates with the formation of acid has long been recognized as one of the characteristics of Bacillus-coli-like bacteria. This property of acid-production is the basis for the isolation of B. coli on the Wurtz litmus-lactose-agar plate, and also for the separation of B. coli from its relatives, B. typhosus and B. paratyphosus, on the Conradi-Drigalski agar medium. The ability to ferment various substances has been further utilized as a basis for the subdivision of the colon-aerogenes group. In these studies on classification, however, attention has been focused upon gas-formation rather than upon acid-production.

Browne,[1] in an extensive study of certain factors influencing acid-production, points out that Bacillus-coli-like bacteria isolated from oysters formed less acid from carbohydrates than those isolated from human stools, and he attributed the difference to a loss of fermenting power by the organisms in their passage through sewage from the intestines to the oysters. Unfortunately, this investigator did not differentiate the different types of organisms with which he was working. It is entirely probable that the smaller amount of acid observed among the oyster strains was due to a greater incidence of some particular type or species of Bacillus-coli-like microorganism rather than to a loss of fermenting power on the part of the intestinal forms. [After the completion of the experimental work for this paper, an article appeared by Clark and Lubs,[2] who pointed out that bovine fecal strains of B. coli give rise to a higher H+-ion concentration in glucose than do nonfecal (grain) strains.]

The present investigation was undertaken to determine the following:

1. Do Bacillus-coli-like organisms from different sources (particularly animal sources) give rise to different amounts of acid in the

[1] Jour. Infect. Dis., 1914, 15, p. 580.
[2] Ibid., 1913, 17, p. 797.

decomposition of fermentable substances, and if they do, are the differences in acid-formation sufficiently great to warrant quantitative acid-production as a reliable differential index?

2. Is quantitative acid-production correlated with (a) the MacConkey types,[3] (b) the Voges-Proskauer reaction, or (c) gas-formation?

3. Are the morphologic and physiologic characteristics correlated with the source?

CULTURES STUDIED

Altogether 167 organisms were studied; 156 were obtained from sewage and from the feces of horse, cow, sheep, pig, and man, and 11 were from the collection of the American Museum of Natural History.

The method of isolation has been described in a previous paper.[4] They were all of the colon-bacillus group; that is, gram-negative, usually short rods, which formed gas from glucose and lactose, coagulated milk, and did not liquefy gelatin in 20 days.

PREPARATION OF MEDIA

The medium employed for tests of acid-production consisted of 1% peptone water to which was added 1% of the test substance. Peptone water, rather than nutrient broth, was used, to eliminate the formation of acid from traces of any other fermentable substance which might be present in beef extract or meat infusion. The reaction of the medium was neutral to phenolphthalein.

Sterilization.—The medium was tubed (10 c.c. in Durham fermentation tubes) and sterilized in the autoclave for 10 minutes at 10 pounds pressure, which is a shorter period than is recommended in the Standard Methods for Water Analysis (1912). Immediately on removal from the autoclave the medium was rapidly cooled by immersion in cold water, then incubated for 2 or 3 days at 37 C. in order to eliminate tubes which had escaped proper sterilization. Nonsterile tubes were rarely found. Sufficient medium was prepared at one time to permit a test of all the cultures on one substance. Variations in the composition of the medium were reduced to a minimum by using distilled water and the same bottle of Witte's peptone throughout the work.

DETERMINATION OF ACID-PRODUCTION

Acid-production was determined in the following manner. A tube of peptone water was inoculated from an agar-slant stock culture and incubated at the body temperature (37 C.) for 24 hours. Two standard 4-mm. loops of this 24-hour peptone culture were then inoculated into each of 2 tubes of peptone medium containing the test substance and incubated for 36 hours at 37 C. Acid-production in duplicate tubes varied so little that duplicates were not employed with dulcitol, galactose, maltose, glycerol, and salicin.

[3] Jour. Hyg., 1905, 5, p. 333; 1909, 9, p. 86.
[4] Levine: Jour. Infect. Dis., 1916, 18, p. 358.

The body temperature was selected for incubation, because, as was shown by Browne,[1] acid-production by B. coli is most rapid at this temperature. He also showed that with certain carbohydrates and alcohols the maximal amount of acid is formed in less than 24 hours. Thirty-six hours' incubation was employed for convenience in this study. With the alcohol, glycerol, and the glucosid, salicin, the 36-hour incubation period was not sufficient. Acid- and gas-formation from these substances were therefore determined after 72 hours' growth.

Titration.—As the acidity of distilled water varied on different days, the following technic was adopted in order to obviate tedious subtractions of checks. To a pail of distilled water (6 to 8 liters) was added 1% phenolphthalein solution (5 gm. phenolphthalein in 1 liter of 50% alcohol). The water was boiled vigorously for 15 minutes and then neutralized with sodium hydroxid. Of this neutral distilled water, containing the indicator, 45 c.c. were dipped out into an evaporating dish or casserole, 5 c.c. of the test culture were added, and the amount of acid determined by titration with N/20 NaOH without boiling.

TREATMENT OF RESULTS

A few extremely high or low results will influence considerably the average acid-production of a collection of organisms. The use of unqualified averages may therefore lead to a misconception of the acid-producing properties of a group. To supplement the arithmetic mean, or numeric average, some statement should be made as to the distribution of the variates about the average. This may be indicated by the probable error or by the standard deviation. The coefficient of variability (the ratio of the standard deviation to the mean) is an excellent abstract measure of variability. The modal acid-production (the amount of acid most frequently formed) may, under certain conditions, be of greater significance than the average amount of acid formed.

In this study the mean, the probable error of a single variate, the standard deviation, the coefficient of variability, and the empirical mode are employed.

The standard deviation is the measure of variability most commonly employed, particularly by mathematicians. It may be expressed mathematically as

$$\sigma = \sqrt{\frac{\Sigma d^2 f}{n}}$$

where "n" is the number of variates, or observations, "d" the deviation of the individual variates from the mean, and "f" the frequency of a deviation "d". The standard deviation serves to indicate whether the departures from the mean are

small or great. The closer the individual organisms group themselves about the mean, or average, the smaller the standard deviation.

An example may make clear the meaning and significance of the standard deviation. Suppose that the amounts of acid formed by a group (A) of 4 organisms in glucose broth are 2.1, 2.2, 2.2, and 2.3% normal acid, and that those formed by another group (B) of 4 organisms are 1.9, 2, 2.4, and 2.5% normal acid. The average for each group is 2.2, but mere inspection shows that the organisms in Group A and those in Group B are quite differently distributed with respect to this average. In large collections of data inspection is impracticable, but the standard deviation serves well in its place. The standard deviation in Group A is ± 0.07 while for Group B it is ± 0.25. The larger deviation in B denotes that the individuals in the group wander farther away from the average than do those in Group A.

The probable error is employed to indicate what confidence is to be placed in statistical results. The reliability of the mean and standard deviation may be determined by calculating their probable errors, but in this paper only the probable error of a single variate is considered. In a normal distribution the probable error of a single variate of a series of observations is defined as that departure from the mean, on either side, within which exactly one-half of the variates are found; that is, if in the study of acid-production by a large number of organisms, it is found that the mean (average) amount of acid formed is 2.25% normal, and that the probable error of a single observation is ± 0.15, then half of the organisms have formed between 2.1% and 2.4% normal acid.

The coefficient of variability is the ratio of the standard deviation to the mean ($\frac{\sigma}{M}$). It is an abstract measure of variability and may therefore be employed to advantage for comparing variability among different characters, or in the same character among different groups of organisms, particularly if their means differ widely.[5]

ACID-PRODUCTION IN SUBSTANCES FERMENTED BY ALL OF THE TEST ORGANISMS

Glucose, galactose, mannitol, maltose, and lactose were decomposed with gas-production by all strains.

A. GLUCOSE

The frequency distributions of the organisms with respect to acid-formation from glucose are shown in Table 1, where the relation of

[5] For a detailed description of these constants the reader is referred to Principles of Breeding (1907), by E. Davenport; Statistical Methods (1904), by C. B. Davenport; Precision of Measurements (1909), by Goodwin, and to an Introduction to the Theory of Statistics (1916), by Yule.

TABLE 1

RELATION OF SOURCE, MACCONKEY TYPE, AND VOGES-PROSKAUER REACTION TO ACID-PRODUCTION IN GLUCOSE

Percentage of Normal Acid	Frequencies												
	MacConkey Type				Source						All Strains	Voges-Proskauer	
	I	II	III	IV	Horse	Sheep	Cow	Pig	Man	Sewage		Negative	Positive
0.00-0.19													
0.20-0.39													
0.40-0.59													
0.60-0.79				1		1					1	1	
0.80-0.99				3		3					3	3	
1.00-1.19				2		1				1	2	1	1
1.20-1.39	3	4	3	1			3	5		3	11	9	2
1.40-1.59	4	3	9	3			5	2	1	11	19	15	4
1.60-1.79	8	5	3	6	2	1	2	7	4	6	22	20	2
1.80-1.99	28	8	10	8	5	5	9	8	14	13	54	54	
2.00-2.19	10	6	17	8	10	11	1	9	5	5	41	41	
2.20-2.39	1			2	2				1		3	3	
Total acid-formers.....	54	26	42	34	19	22	20	31	25	39	156	147	9
Mode.....	1.90	1.90	2.10	2.00	2.10	2.10	1.90	2.10	1.90	1.90	1.90	1.90	1.50
Mean.....	1.85	1.77	1.84	1.71	2.03	1.76	1.70	1.79	1.91	1.71	1.80	1.82	1.46
Probable error	±.14	±.18	±.19	±.29	±.11	±.32	±.17	±.18	±.11	±.18	±.20	±.20	±.12
Standard deviation.....	±.21	±.27	±.28	±.43	±.16	±.47	±.25	±.27	±.17	±.26	±.30	±.30	±.18
Coefficient of variation....	11.3	15.2	15.2	25.1	7.9	26.7	14.7	15.1	8.9	15.2	16.6	16.5	12.3

acid-production to the source, to the MacConkey types, and to the Voges-Proskauer reaction is also indicated.

The mode for acid-production by all strains is at 1.9% normal, with the mean at 1.8% normal acid.

The means or average quantities of acid formed by the MacConkey types indicate that Types III (communior) and I (acidi-lactici) produce about equal quantities of acid (1.84 and 1.85% normal respectively), while Type II (communis) forms somewhat less (1.77%), and Type IV (aerogenes) the least amount (1.71%). A comparison of Type IV, which forms the smallest quantity of acid, with Type I, which gives the greatest amount, indicates that the means tend to exaggerate the difference between the two types in ability to form acid from glucose. Type I has a well-defined mode at 1.90% and Type IV has a very indistinct mode at about the same point. The standard deviation in Type IV is ±0.43, or three times as great as the difference between the means of the two MacConkey types. Similar observations

may be made on the other types. It is therefore apparent that quantitative acid-production in glucose is not a reliable criterion for differentiation of the MacConkey types.

There are many irregularities in the frequency distributions of organisms from different sources with respect to acid-production in glucose. The organisms from horse and man group themselves in a manner simulating a normal distribution, but the frequency curves of those from cow, sheep, and pig, contain 2 modes. These multiple modes are probably due to the choice of classes and to the small number of cultures from each source. In the other test substances multiple modes are very infrequent. In the column headed "Mode," in the frequency tables, the primary mode is recorded.

The distribution of organisms from the sheep is interesting. Two distinct groups are indicated, one of which generally produces more than 2% normal acid and the other usually less than 1%. Of the 5 low-acid-formers, 4 are from a single animal (all the cultures obtained from that animal), and they are distinguished morphologically from all the other sheep strains in that they are distinctly longer.

Among the sewage strains 2 well-defined modes are evident, at 1.9% and 1.5% normal acid, corresponding with the modes of the Voges-Proskauer-negative and the Voges-Proskauer-positive organisms respectively.

In a consideration of the different animal sources it appears that the average amount of acid formed from glucose by Bacillus-coli-like organisms from horse (2.03%) is greater than the amounts formed by strains from pig, sheep and cow (1.70, 1.76, and 1.79%), while the amount formed by human strains is intermediate (1.91% normal). This relationship does not hold for other test substances and there does not seem to be any marked relation between the quantities of acid produced from glucose, and those formed from other fermentable carbohydrates or alcohols by colon-bacillus-like organisms from the animals recorded here.

Quantitative acid-formation is better correlated with the Voges-Proskauer reaction than with the source or with MacConkey's groups. The Voges-Proskauer-negative organisms give an average of 1.82% normal acid, with a mode at 1.90%, while the Voges-Proskauer-positive strains 1.46%, with a mode at 1.50% normal, and, altho the difference, 0.36, is probably not sufficient for reliable differentiation, it is nevertheless significant, because rather striking differences in acid-formation between the Voges-Proskauer-positive and the Voges-Pros-

kauer-negative strains are observed with many other test substances, as maltose, sucrose, glycerol, and dulcitol.

B. GALACTOSE

The frequency distributions with respect to acid-production in galactose are shown in Table 2. Less acid is formed from galactose than from glucose, and the frequency distributions are very nearly normal. Multiple modes are not present. The average amount of

TABLE 2

RELATION OF SOURCE, MACCONKEY TYPE, AND VOGES-PROSKAUER REACTION TO ACID-PRODUCTION IN GALACTOSE

Percentage of Normal Acid	Frequencies												
	MacConkey Type				Source						All Strains	Voges-Proskauer	
	I	II	III	IV	Horse	Sheep	Cow	Pig	Man	Sewage		Negative	Positive
0.00-0.19													
0.20-0.39				1			1				1	1	
0.40-0.59													
0.60-0.79				1						1	1	1	
0.80-0.99				2		1				1	2	1	1
1.00-1.19	3		4	6		3	1	2	1	6	13	8	5
1.20-1.39	28	18	21	10	8	6	12	17	19	15	77	76	1
1.40-1.59	21	8	16	13	11	11	6	11	5	14	58	57	1
1.60-1.79	1		1							2	2	1	1
1.80-1.99													
2.00-2.19													
Total acid-formers.....	53	26	42	33	19	21	19	30	25	39	154	145	9
Mode.....	1.30	1.30	1.30	1.50	1.50	1.50	1.30	1.30	1.30	1.30	1.30	1.30	1.10
Mean.....	1.37	1.36	1.37	1.27	1.42	1.36	1.35	1.36	1.33	1.34	1.35	1.36	1.21
Probable error	±.06	±.06	±.09	±.18	±.07	±.12	±.07	±.08	±.06	±.14	±.12	±.11	±.16
Standard deviation.....	±.12	±.09	±.13	±.27	±.10	±.18	±.11	±.12	±.09	±.20	±.17	±.16	±.28
Coefficient of variation....	8.8	6.6	9.5	21.2	7.1	13.2	8.2	8.8	6.8	14.9	12.6	11.8	19.0

acid formed by all strains is 1.35% normal, with a distinct mode at 1.30%. (Acid was not determined from 2 cultures, 1 from pig and 1 from sheep, which broke just before titration.)

The MacConkey types, I, II, and III, each have a mode at 1.30% normal acid, and means at 1.37, 1.36, and 1.37% normal acid respectively. Altho the mode for Type IV is 1.50% normal acid—somewhat higher than for the other types—the mean, 1.27%, is lower, a circumstance indicating a greater variability in Type IV. This greater

variability is indicated by the much larger standard deviation and coefficient of variability. MacConkey types, therefore, cannot be differentiated on the basis of quantitative acid-production in galactose as indicated by titration with phenolphthalein.

There does not seem to be any correlation between the amount of acid formed from galactose and the source of the organisms. One organism from the cow formed less than 0.4% acid. It was omitted in calculating acid-production by the group. If included, the mean for the cow strains becomes 1.30%, with a coefficient of variability of 19.2%.

The Voges-Proskauer-positive strains form somewhat less acid (1.21%) than do the Voges-Proskauer-negative strains (1.36%). The difference (0.15% normal) is slight, but it is greater than the differences observed with the MacConkey types or with the strains from different sources. The difference is of some interest, moreover; for, as will appear later, whereas the Voges-Proskauer-positive strains form less acid from the monosaccharids than do the Voges-Proskauer-negative strains, the reverse is true when more complex substances (except lactose) are fermented.

C. MANNITOL

The hexite, mannitol, is attacked about as readily as galactose. The average amount of acid formed by all strains is 1.32%, with a sharp mode at 1.30% normal. (Acid-production was not determined in 2 cultures, 1 from horse and 1 from man.) The frequency distributions and the relation of the Voges-Proskauer reaction, the source, and the MacConkey types to the amount of acid formed from mannitol are shown in Table 3.

The mode for each of the MacConkey types is at 1.30%, and the means are 1.32, 1.30, 1.33, and 1.31% normal acid respectively. The MacConkey types are therefore indistinguishable on the basis of quantitative acid-production from mannitol.

A comparison of the amounts of acid formed by Voges-Proskauer-negative and Voges-Proskauer-positive strains indicates that the latter attack mannitol somewhat more readily, but the difference is not appreciable.

Except for the horse strains, which have a mode at 1.50%, the organisms from all the other sources group themselves around 1.30% normal acid as a mode. In general, the differences observed between the means are too slight to be of any significance. The average of the

sheep strains is the lowest, 1.21%, as compared with 1.34% for human strains, 1.38% for horse, 1.36% for sewage, 1.31% for cow, and 1.28% normal for pig strains. The lower average of the sheep strains is due to the presence among them of a few low-acid-producing organisms rather than to a lesser ability of the group as a whole to attack mannitol. That sheep strains form acid from mannitol as readily as do those from the cow, pig, and man is indicated by the coincidence of their

TABLE 3

RELATION OF SOURCE, MACCONKEY TYPE, AND VOGES-PROSKAUER REACTION TO ACID-PRODUCTION IN MANNITOL

Percentage of Normal Acid	Frequencies												
	MacConkey Type				Source						All Strains	Voges-Proskauer	
	I	II	III	IV	Horse	Sheep	Cow	Pig	Man	Sewage		Negative	Positive
0.00-0.19													
0.20-0.39													
0.40-0.59													
0.60-0.79				2		2					2	2	
0.80-0.99	1			4	1	3		1			5	5	
1.00-1.19	9	6	7		1	3	5	6	3	4	22	22	
1.20-1.39	29	14	20	15	6	9	9	19	13	22	78	75	3
1.40-1.59	13	6	14	12	10	5	6	5	8	11	45	40	5
1.60-1.79	1									1	1	1	
1.80-1.99				1						1	1		1
2.00-2.19													
Total acid-formers.....	53	26	41	34	18	22	20	31	24	39	154	145	9
Mode.....	1.30	1.30	1.30	1.30	1.50	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.50
Mean.....	1.32	1.30	1.33	1.31	1.38	1.21	1.31	1.28	1.34	1.36	1.32	1.31	1.48
Probable error	±.10	±.09	±.09	±.17	±.11	±.18	±.10	±.09	±.09	±.12	±.12	±.12	±.12
Standard deviation.....	±.15	±.14	±.14	±.26	±.17	±.26	±.15	±.14	±.13	±.18	±.18	±.18	±.18
Coefficient of variation....	11.3	10.8	10.5	19.8	12.3	21.2	11.4	10.9	9.7	13.2	13.6	13.7	12.1

modes. The strains from the horse tend to form somewhat more acid than do those from other animals, but the difference is too slight to be of any differential significance.

D. LACTOSE

The amount of acid formed from the disaccharid, lactose, in 1% lactose peptone solution is very nearly the same as that formed from the monosaccharid, galactose, and from the hexite, mannitol. The mode for all strains is at 1.30%, and the mean is also at 1.30% normal acid. The frequency distributions are shown in Table 4.

MacConkey Type II has an ill-defined mode at 1.50%, and the mean is 1.25% normal acid. The mode for the other types (I, III, IV) is at 1.30%, and the means are 1.31, 1.32, and 1.33% normal acid respectively. The MacConkey types are indistinguishable on the basis of quantitative acid-production in lactose.

There is no evident relation between the source and the amount of acid produced from lactose.

TABLE 4

RELATION OF SOURCE, MACCONKEY TYPE, AND VOGES-PROSKAUER REACTION TO ACID-PRODUCTION IN LACTOSE

Percentage of Normal Acid	Frequencies												
	MacConkey Type				Source						All Strains	Voges-Proskauer	
	I	II	III	IV	Horse	Sheep	Cow	Pig	Man	Sewage		Negative	Positive
0.00-0.19													
0.20-0.39		1					1				1	1	
0.40-0.59													
0.60-0.79		3					3				3	3	
0.80-0.99	1	1		6		5	1			2	8	7	1
1.00-1.19	13	1	11		4	1	1	6	12	1	25	24	1
1.20-1.39	30	9	19	15	10	3	11	18	11	20	73	67	6
1.40-1.59	8	11	10	10	5	9	3	7	2	13	39	38	1
1.60-1.79			2	3		4				1	5	5	
1.80-1.99	2									2	2	2	
2.00-2.19													
Total acid-formers.....	54	26	42	34	19	22	20	31	25	39	156	147	9
Mode.....	1.30	1.50	1.30	1.30	1.30	1.50	1.30	1.30	1.30	1.30	1.30	1.30	1.30
Mean.....	1.30	1.25	1.31	1.32	1.31	1.25	1.16	1.31	1.22	1.38	1.30	1.31	1.26
Probable error	±.13	±.22	±.11	±.16	±.09	±.19	±.22	±.09	±.09	±.13	±.15	±.15	±.11
Standard deviation.....	±.17	±.32	±.17	±.23	±.14	±.28	±.33	±.13	±.13	±.20	±.22	±.22	±.16
Coefficient of variation....	13.1	25.6	13.0	17.4	10.7	22.4	28.4	10.0	10.6	14.5	16.9	16.8	12.7

There is no distinction between the amounts of acid produced from lactose by Voges-Proskauer-positive and Voges-Proskauer-negative strains. Both groups have their modes at 1.30%, and the means are but slightly removed from the modes, being 1.26 and 1.31% normal acid respectively.

E. MALTOSE

Decomposition of the disaccharid, maltose, yields considerably less acid than the decomposition of the monosaccharids, glucose and galactose, the hexite, mannitol, or the disaccharid, lactose, mentioned. All

strains considered, the average acid-production was 0.77%, with a very distinct mode at 0.7% normal acid. The frequency distributions of the organisms from different sources, of the MacConkey types, and of the Voges-Proskauer reaction with respect to acid-production in maltose are shown in Table 5. One organism apparently fails to show acid but forms gas. This neutral reaction is presumed to be due to reversion.

TABLE 5

RELATION OF SOURCE, MACCONKEY TYPE, AND VOGES-PROSKAUER REACTION TO ACID-PRODUCTION IN MALTOSE

Percentage of Normal Acid	Frequencies												
	MacConkey Type				Source						All Strains	Voges-Proskauer	
	I	II	III	IV	Horse	Sheep	Cow	Pig	Man	Sewage		Negative	Positive
0.00-0.19		1								1	1	1	
0.20-0.39													
0.40-0.59	7	2	6	1		3		3	6	4	16	16	
0.60-0.79	31	17	24	18	9	19	13	15	18	16	90	90	
0.80-0.99	10	6	8	9	9		8	12	1	8	33	31	2
1.00-1.19	6		2	4	1		4	1		6	12	8	4
1.20-1.39			2	2						4	4	1	3
1.40-1.59													
1.60-1.79													
1.80-1.99													
2.00-2.19													
Total acid-formers.....	54	25	42	34	19	22	20	31	25	33	155	146	9
Mode.....	0.70	0.70	0.70	0.70	0.80	0.70	0.70	0.70	0.70	0.70	0.70	0.70	1.10
Mean.....	0.76	0.73	0.76	0.83	0.81	0.67	0.81	0.77	0.67	0.85	0.77	0.75	1.12
Probable error	±.11	±.07	±.13	±.13	±.08	±.05	±.11	±.09	±.08	±.15	±.11	±.09	±.10
Standard deviation.....	±.16	±.11	±.19	±.19	±.11	±.07	±.16	±.14	±.12	±.23	±.17	±.14	±.15
Coefficient of variation....	21.0	15.1	25.0	22.9	13.6	10.4	19.8	18.2	17.9	27.0	22.1	18.7	13.4

Each of the MacConkey types has a sharply defined mode at 0.70% normal. The means for the types are 0.76, 0.73, 0.76, and 0.83% normal acid respectively. There is no correlation between quantitative acid-formation from maltose and the MacConkey types.

The relation of acid-formation from maltose to the source of Bacillus-coli-like organisms is not at all striking. The mode for each source is at 0.7% normal acid. The trend of the variation among the strains from horse, cow, pig, and sewage, is beyond the mode, so that the means are 0.81, 0.81, 0.77, and 0.83% normal acid respec-

tively, while the strains from man and sheep vary in the other direction, lowering the averages to 0.67% for man and 0.68% for sheep. It should be pointed out that the relatively high average for sewage, 0.83%, is due to the presence of Voges-Proskauer-positive organisms. The average for the sewage strains exclusive of the Voges-Proskauer-positive organisms, is 0.73% normal acid. Acid-production in maltose can not be considered a reliable index for differentiation of Bacillus-coli-like organisms from the sources studied.

There is a rather marked and distinct relation between quantitative acid-production in maltose and the Voges-Proskauer reaction. It appears from Table 5 that the Voges-Proskauer-negative strains occasionally form more than 1% acid, but usually less than 0.8%, while the Voges-Proskauer-positive strains usually form more than 1% and never less than 0.8% normal acid. The mode for the Voges-Proskauer-negative strains is at 0.70% and the mean at 0.78% normal acid. The mode and mean for the Voges-Proskauer-positive strains are 1.10 and 1.12% respectively.

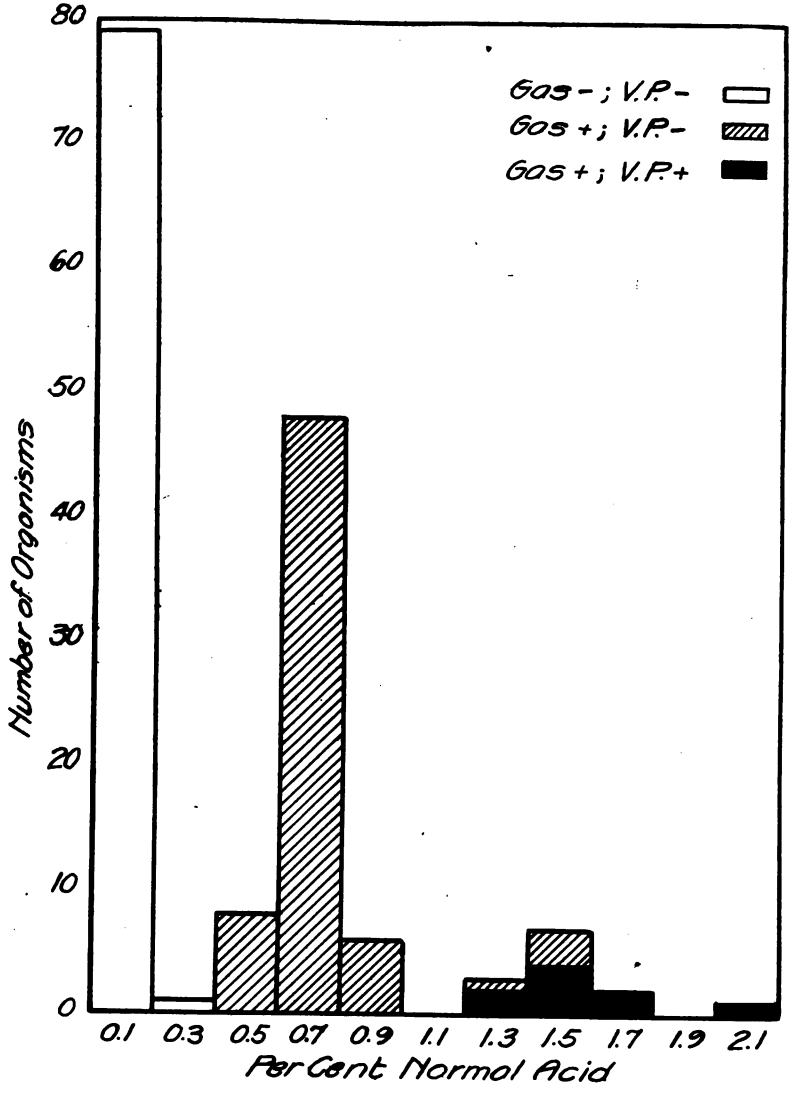
ACID-PRODUCTION IN SUBSTANCES NOT FERMENTED BY ALL THE TEST ORGANISMS

The disaccharid, sucrose, the trisaccharid, raffinose, the glucosid, salicin, and the alcohols, glycerol and dulcitol, were attacked by many, but not by all, of the organisms studied.

In calculating means and other constants for acid-formation, only those organisms which attacked the test substances were included. The line of demarcation for acid-production was selected at 0.4% normal acid, because in sucrose, raffinose, and dulcitol organisms which formed less than this amount in 36 hours at 37 C. rarely, if ever, formed gas, while those which produced more than 0.4% acid, practically always formed gas also.

A. SUCROSE

In Table 6 are shown the frequency distributions of acid-production in sucrose in relation to the MacConkey types, the source, and the Voges-Proskauer reaction. The relation of acid-production to gas-formation, and to the Voges-Proskauer reaction, is indicated, also, in Plot 1. (One organism, which was overrun in titration, is not included in the calculation.) Three modes are evident. One mode is at 0.1% normal acid and represents those organisms which do not form gas from sucrose. Acid-formation and gas-production in suc-



PLOT I

ACID-PRODUCTION FROM SUCROSE (All Strains)

-ose are well correlated. Colon-bacilli-like organisms which form gas, also give rise to acid and vice versa. Among the gas-formers 2 groups are apparent; one forms acetylmethylcarbinol from glucose (V.P.+) and a relatively large amount of acid from sucrose (mode at 1.50% normal), while the other does not form acetylmethylcarbinol from glucose (V.P.—) and gives rise to a much smaller quantity of acid from sucrose (an extremely sharp and distinct mode at 0.70% normal).

MacConkey Types I and II do not form acid from sucrose. That Types III and IV are indistinguishable on the basis of quantitative acid-production in sucrose, is apparent from Table 6.

Ten of the organisms from cow, 15 from horse, 20 from sheep, 10 from pig, and only 3 from man, ferment sucrose. The amount of acid formed bears no definite relation to the animal source, but it should

TABLE 6

RELATION OF SOURCE, MACCONKEY TYPE, AND VOGES-PROSKAUER REACTION TO ACID-PRODUCTION IN SUCROSE

Percentage of Normal Acid	Frequencies												
	MacConkey Type				Source						All Strains	Voges-Proskauer	
	I	II	III	IV	Horse	Sheep	Cow	Pig	Man	Sewage		Negative	Positive
0.00 0.19	53	26			4	1	10	21	21	22	79	79	
0.20-0.39	1								1		1	1	
0.40-0.59			3	5		3		2		3	8	8	
0.60-0.79			30	18	10	16	9	7	3	3	48	48	
0.80-0.99			4	2	2	1	1	1		1	6	6	
1.00-1.19													
1.20-1.39			1	2	1					2	3	1	2
1.40-1.59			3	4	2					5	7	3	4
1.60-1.79			1	1						2	2		2
1.80-1.99													
2.00-2.19				1						1	1		1
Total acid-formers.....	....	....	42	33	15	20	10	10	3	17	75	66	9
Mode.....	....	....	0.70	0.70	0.70	0.70	0.70	0.70	0.70	1.50	0.70	0.70	1.50
Mean.....	....	....	0.80	0.91	0.93	0.68	0.72	0.68	0.70	1.18	0.84	0.74	1.57
Probable error			±.18	±.27	±.20	±.06	±.04	±.07		±.33	±.23	±.14	±.16
Standard deviation.....			±.27	±.40	±.29	±.09	±.06	±.11		±.49	±.34	±.20	±.23
Coefficient of variation....	....	...	33.8	44.0	31.2	13.2	8.3	16.2		41.5	40.5	27.0	14.6

be noted that a few cultures among the horse strains form considerably more acid than any of the other animal strains. The high average for

the horse strains is due to the influence of these few cultures and is not a characteristic of horse strains in general.

The high average, 1.18% normal acid, of the 17 sewage strains which attacked sucrose, is due entirely to the presence among them of 9 Voges-Proskauer-positive organisms. The mean for the other 8 sewage strains is 0.75% normal acid.

Voges-Proskauer-negative strains attack sucrose less readily than the Voges-Proskauer-positive strains. The means for the two groups are 0.74 and 1.57%, and the empirical modes 0.7 and 1.5% normal acid respectively.

B. RAFFINOSE

The frequency distributions of the organisms with respect to acid-production in raffinose and the relation of acid-formation to the MacConkey types, to the source, and to the Voges-Proskauer reaction, are given in Table 7.

TABLE 7

RELATION OF SOURCE, MACCONKEY TYPE, AND VOGES-PROSKAUER REACTION TO ACID-PRODUCTION IN RAFFINOSE

Percentage of Normal Acid	Frequencies												
	MacConkey Type				Source						All Strains	Voges-Proskauer	
	I	II	III	IV	Horse	Sheep	Cow	Pig	Man	Sewage		Negative	Positive
0.00-0.19	50	23			3		8	20	21	21	73	73	
0.20-0.39		1					1				1	1	
0.40-0.59	1		2	1	1	1		1		1	4	4	
0.60-0.79			13	5	5	4	2	5		2	18	18	
0.80-0.99	2	1	10	5	3	3	6	2	2	2	18	18	
1.00-1.19	1		8	11	4	3	1	2	1	4	20	19	1
1.20-1.39		1	8	10	3	5	2	1	1	7	19	13	6
1.40-1.59			1	2		1				2	3	1	2
1.60-1.79													
1.80-1.99													
2.00-2.19													
Total acid-formers.....	4	2	42	34	16	22	11	11	4	18	82	78	9
Mode.....	....	....	0.70	1.10	0.70	1.10	0.90	0.70	0.90	1.30	1.10	1.10	1.30
Mean.....	....	....	0.95	1.08	0.94	1.03	0.97	0.85	1.05	1.11	1.00	0.96	1.32
Probable error			±.17	±.17	±.15	±.17	±.12	±.15	±.11	±.19	±.17	±.16	±.08
Standard deviation.....			±.25	±.25	±.22	±.25	±.18	±.22	±.17	±.28	±.26	±.24	±.11
Coefficient of variation....	....	....	26.3	22.9	23.4	24.3	18.6	25.9	16.2	25.2	26.0	25.5	8.3

All the strains considered, somewhat more acid is formed from raffinose (1% normal) than from sucrose (0.7%). No distinct mode

is present, and the dispersion of the distribution is very great, as indicated by a large coefficient of variability (26%).

MacConkey Types I and II generally do not attack raffinose. The few strains in these types which do, are not sufficient for comparative purposes. Type IV tends to form more acid than Type III, but the difference is not considered a reliable index for differentiation.

There is no apparent relation between the animal source and the amount of acid formed from raffinose. The mean for the sewage strains, 1.11% normal acid, is higher than for those from other sources. This difference, as was observed in the case of sucrose, is due to the presence of the Voges-Proskauer-positive group among the sewage strains. The mean for the Voges-Proskauer-negative strains in sewage is 0.94% normal acid.

The Voges-Proskauer-negative strains attack raffinose less readily than do the Voges-Proskauer-positive strains. The means for the two groups are 0.96 and 1.32% normal acid respectively, but the variability among the strains is such as to make the difference (0.36) of questionable significance.

C. GLYCEROL

The alcohol, glycerol, is attacked by many strains which form acid but not gas. For calculating acid-production all strains which form more than 0.4% normal acid in 72 hours at 37 C. are regarded as acid-formers. The frequency distributions and the relation of quantitative acid-production to the MacConkey types, to the source, and to the Voges-Proskauer reaction are shown in Table 8. A sharp mode is observed at 0.70% and the mean for all the strains is at 0.73% normal acid.

The MacConkey types are indistinguishable on the basis of quantitative acid-production in 1% glycerol peptone solution altho Type III tends to form somewhat less acid than the others.

The differences between the means of organisms from the various animal sources cannot be regarded as significant. The sheep strains form the least amount of acid (0.60% normal), while the means for strains from other sources are, horse 0.67%, cow 0.71%, man 0.71%, and pig 0.76% normal acid. The somewhat higher mean of the sewage strains (0.83%) is due, again, to the influence of Voges-Proskauer-positive strains. These eliminated, the mean for the Voges-Proskauer-negative strains in sewage is 0.70% normal.

One of the Voges-Proskauer-positive strains does not attack glycerol. This is probably B. cloacae. Those Voges-Proskauer-positive organisms which do ferment glycerol, generally form much more acid than the Voges-Proskauer-negative fermenting strains. The mean for the Voges-Proskauer-negative organisms coincides with the mode at 0.70% normal. The Voges-Proskauer-positive organisms have a mode at 1.50%, with a mean at 1.28% normal acid.

TABLE 8

RELATION OF SOURCE, MACCONKEY TYPE, AND VOGES-PROSKAUER REACTION TO ACID-PRODUCTION IN GLYCEROL

Percentage of Normal Acid	Frequencies												
	MacConkey Type				Source						All Strains	Voges-Proskauer	
	I	II	III	IV	Horse	Sheep	Cow	Pig	Man	Sewage		Negative	Positive
0.00-0.19		1	1	2	1					3	4	3	1
0.20-0.39	2		2	1		2		2		1	5	5	
0.40-0.59	10	1	20	8	8	10	2	4	7	8	39	39	
0.60-0.79	25	16	13	12	5	9	15	13	13	11	66	66	
0.80-0.99	16	8	3	6	5		3	12	3	10	33	31	2
1.00-1.19	1			1					1	1	2	1	1
1.20-1.39				2					1	1	2	1	1
1.40-1.59			3	1						4	4		4
1.60-1.79													
1.80-1.99													
2.00-2.19													
Total acid-formers.....	52	25	39	30	18	19	20	29	25	35	146	138	8
Mode.....	0.70	0.70	0.50	0.70	0.50	0.50	0.70	0.70	0.70	0.70	0.70	0.70	1.50
Mean.....	0.73	0.76	0.67	0.77	0.67	0.60	0.71	0.76	0.71	0.83	0.73	0.70	1.28
Probable error	±.10	±.07	±.17	±.17	±.11	±.06	±.07	±.09	±.13	±.20	±.14	±.11	±.16
Standard deviation.....	±.15	±.10	±.25	±.25	±.17	±.09	±.10	±.14	±.19	±.30	±.21	±.16	±.24
Coefficient of variation....	20.5	13.2	37.4	32.5	25.4	15.0	14.1	18.4	26.8	36.2	28.8	24.3	16.0

D. DULCITOL

In Table 9 is indicated the relation of the MacConkey types, the source, and the Voges-Proskauer reaction to acid-production in dulcitol.

MacConkey Types I and IV do not form acid from dulcitol. Types II and III produce about equal quantities, 0.88% and 0.81% normal acid respectively, but Type II shows a greater variability.

The sheep, pig, human, and horse strains attack dulcitol more vigorously than do the organisms from the cow. The averages are,

TABLE 9

RELATION OF SOURCE, MACCONKEY TYPE, AND VOGES-PROSKAUER REACTION TO ACID-PRODUCTION IN DULCITOL

Percentage of Normal Acid	Frequencies												
	MacConkey Type				Source						All Strains	Voges-Proskauer	
	I	II	III	IV	Horse	Sheep	Cow	Pig	Man	Sewage		Negative	Positive
0.00-0.19	53			33	6	11	9	18	19	23	86	80	6
0.20-0.39		1	1	1	2		1				3	3	
0.40-0.59	1	5				1	2		1	2	6	6	
0.60-0.79		7	16		5	1	5	4	2	6	23	23	
0.80 0.99		6	21		6	9	3	4	1	4	27	27	
1.00-1.19		6	1					5	2		7	7	
1.20-1.39			3							3	3		3
1.40-1.59													
1.60-1.79													
1.80-1.99													
2.00-2.19													
Total acid-formers.....	1	24	41		11	11	10	13	6	15	66	63	3
Mode.....	....	0.70	0.90		0.90	0.90	0.70	1.10		0.70	0.90	0.90	1.30
Mean.....	....	0.81	0.88		0.81	0.84	0.72	0.92	0.83	0.85	0.88	0.81	1.30
Probable error		±.15	±.13		±.07	±.08	±.09	±.11	±.15	±.18	±.15	±.11	
Standard deviation.....		±.22	±.20		±.10	±.12	±.14	±.16	±.22	±.26	±.22	±.17	
Coefficient of variation....	....	27.2	22.7		12.3	14.3	19.4	17.4	26.5	30.6	26.5	21.0	

pig 0.92%, sheep 0.84%, human 0.83%, and horse 0.81%, as compared with 0.72% normal acid for cow. The number of fermenting strains from the different sources is too small for reliable comparison and the differences here indicated are insignificant.

Only 3 of the Voges-Proskauer-positive strains ferment dulcitol, but the amount of acid produced by each of these three organisms is greater than that formed by any of the Voges-Proskauer-negative strains. The mean for the Voges-Proskauer-positive organisms is 1.30%, and for the Voges-Proskauer-negative cultures 0.81% normal acid.

E. SALICIN

Acid-production in salicin, as in glycerol, is not always accompanied by gas-formation. The frequency distribution with respect to source, MacConkey type, and Voges-Proskauer reaction is indicated in Table 10.

TABLE 10

RELATION OF SOURCE, MACCONKEY TYPE, AND VOGES-PROSKAUER REACTION TO ACID-PRODUCTION IN SALICIN

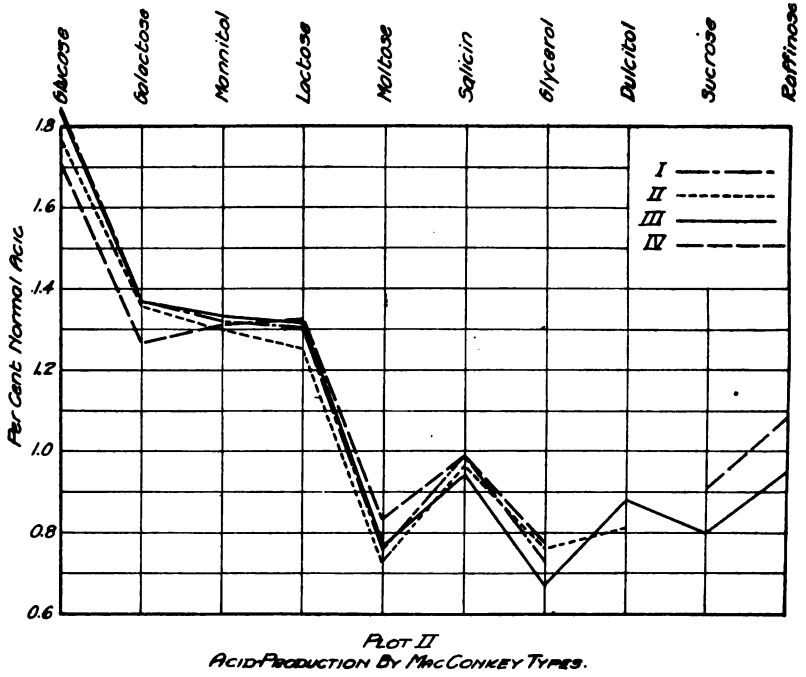
Percentage of Normal Acid	Frequencies												
	MacConkey Type				Source						All Strains	Voges-Proskauer	
	I	II	III	IV	Horse	Sheep	Cow	Pig	Man	Sewage		Negative	Positive
0.00-0.19	26	1	8	8	6	7	1	8	13	8	43	43	
0.20-0.39				1			1				1	1	
0.40-0.59	1			3				2	1	1	4	3	1
0.60-0.79	6	5	10	4	3	6	3	5	4	4	25	25	
0.80-0.99	8	9	16	7	5	6	10	7	5	7	40	40	
1.00-1.19	7	9	4	5	2	3	3	7	2	8	25	23	2
1.20-1.39	6	2	1	4	3		2	2		6	13	11	2
1.40-1.59			2	1						3	3		3
1.60-1.79				1						1	1		1
1.80-1.99			1							1	1	1	
2.00-2.19													
Total acid-formers.....	28	25	34	25	13	15	18	23	12	31	112	103	9
Mode.....	0.90	1.00	0.90	0.90	0.90	0.90	0.90	1.00	0.90	1.10	0.90	0.90	1.50
Mean.....	0.98	0.96	0.94	0.98	0.98	0.94	0.94	0.92	0.83	1.11	0.96	0.94	1.28
Probable error	±.16	±.12	±.17	±.21	±.15	±.07	±.12	±.15	±.12	±.21	±.16	±.15	±.22
Standard deviation.....	±.23	±.17	±.26	±.30	±.22	±.11	±.17	±.22	±.17	±.31	±.23	±.22	±.33
Coefficient of variation....	23.5	17.7	27.7	30.6	22.5	11.7	18.1	23.9	20.5	27.9	23.9	23.4	25.8

Inspection of Table 10 shows that the MacConkey types cannot be differentiated on the basis of the amount of acid formed from salicin. Neither is quantitative acid-production an index to the animal source. The Voges-Proskauer-positive strains give more acid (1.28% normal) than the Voges-Proskauer-negative strains (0.94% normal), but the difference is not as marked or distinct as with sucrose and should not be regarded as a differential index.

RÉSUMÉ

A study of the quantities of acid formed by Bacillus-coli-like organisms from different sources (pig, cow, sheep, horse, man, and sewage) when they are inoculated into peptone water containing 1% of various fermentable substances, indicates the following:

1. The MacConkey types are indistinguishable on the basis of quantitative acid-production in the fermentable carbohydrates, the alcohols, and the glucosid studied. This is shown in Plot II, in which

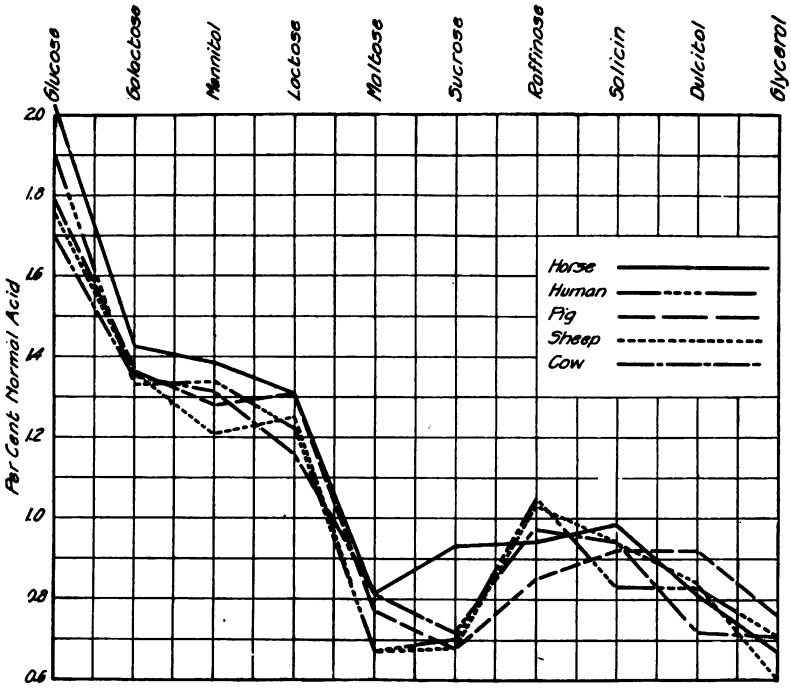


PLOT II
ACID-PRODUCTION BY MACCONKEY TYPES.

the curves for the different types run almost parallel and very close together.

2. That there is no correlation between the amount of acid formed from the carbohydrates, the alcohols, and the glucosid studied, and the animal source, is apparent from Plot III, in which a parallelism similar to that in Plot II is observed. The high average of acid-formation in sucrose among the horse strains is due to the presence among them of a few high-acid-producing cultures, not to any ability of horse strains, as a whole, to yield more acid from sucrose. This has been previously indicated in Table 6.

3. In a general way, the Voges-Proskauer-positive strains isolated from sewage form less acid from the monosaccharids, but more acid from the more complex carbohydrates, etc., (except lactose) than the Voges-Proskauer-negative strains. That this difference is not peculiar to the strains isolated for this study, but is characteristic of the Voges-Proskauer-positive and -negative strains in general, is indicated by the



PLOT III
ACID-PRODUCTION BY COLI-LIKE BACTERIA FROM ANIMAL FECES

similar results obtained with the 11 cultures from the collection of the American Museum of Natural History. Four of the museum strains were positive and 7 negative for the Voges-Proskauer reaction.

The average quantities of acid formed from different substances by the Voges-Proskauer-positive and -negative strains obtained from the museum collection and isolated in this laboratory are shown in Table 11 and Plot IV.

The museum strains, both Voges-Proskauer-positive and -negative, form less acid from lactose than the organisms freshly isolated from animals and sewage, but in all other substances tested the differences between the museum and freshly isolated strains are inappreciable. To infer that the museum strains have lost their power to ferment lactose does not offer an adequate explanation of all the phenomena, for it becomes necessary to explain why the organisms should single out and taboo lactose while retaining their power to form acid from the simpler and more easily attacked monosaccharids, as well as the more

difficultly fermented disaccharids, trisaccharid, alcohols, and glucosid studied. No attempt will therefore be made to explain this phenomenon with lactose, except to suggest that it may possibly be attributed to the small number of museum strains studied.

An inspection of Plot IV and Table 11 indicates that all the 167 strains studied considered, the Voges-Proskauer-positive organisms form less acid from glucose than do the Voges-Proskauer-negative strains, and about equal quantities from galactose, mannitol, and lactose. In all other test substances—maltose, salicin, raffinose, dulcitol, glycerol, and sucrose—the Voges-Proskauer-positive strains give rise to more acid, the excess increasing in the order named. The differ-

TABLE 11

ACID-PRODUCTION IN FERMENTABLE SUBSTANCES BY VOGES-PROSKAUER-POSITIVE AND -NEGATIVE BACILLUS-COLI-LIKE BACTERIA

Test Substance	Percentage of Normal Acid				Excess of Acid (in Percentage of Normal) by the V. P.+ Organisms	
	American-Museum Strains		Levine's Strains			
	V. P. —	V. P. +	V. P. —	V. P. +	American-Museum Strains	Levine's Strains
Glucose	1.82	1.52	1.82	1.46	— .30	— .36
Galactose	1.31	1.28	1.36	1.21	— .03	— .15
Lactose	0.96	0.85	1.31	1.26	— .11	— .05
Mannitol	1.37	1.41	1.31	1.48	+ .04	+ .17
Maltose	0.66	1.01	0.75	1.12	+ .35	+ .37
Sucrose	0.71	1.52	0.74	1.57	+ .81	+ .83
Raffinose	0.79	1.22	0.96	1.32	+ .43	+ .36
Glycerol	0.58	1.27	0.70	1.28	+ .69	+ .58
Dulcitol	0.83	1.15	0.81	1.30	+ .32	+ .49
Salicin	1.00	1.38	0.94	1.28	+ .38	+ .34

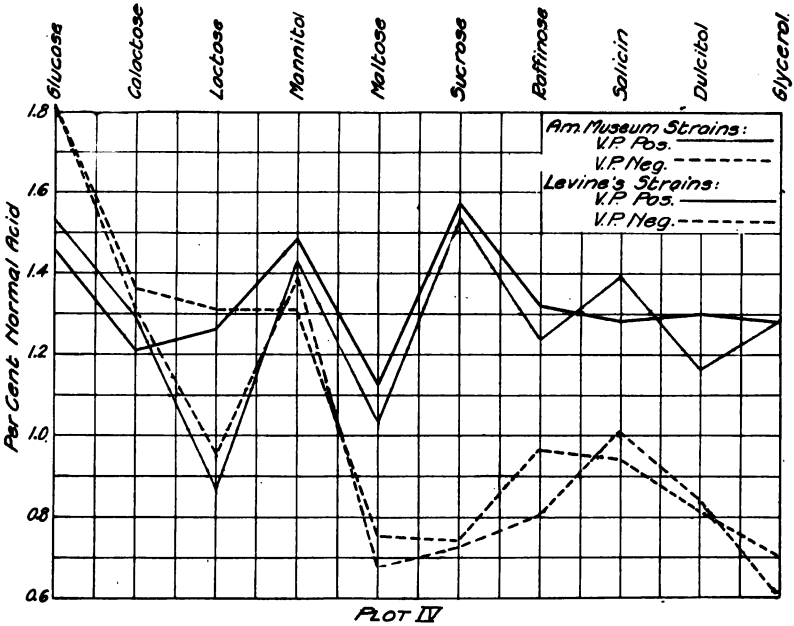
ences obtained in salicin, raffinose, and possibly glucose, are probably not significant, on account of the variations observed in acid-production from these substances.

THE SUPPOSED LOSS OF FERMENTING POWER BY B. COLI IN ITS PASSAGE THROUGH SEWAGE

Browne observed that colon-bacillus-like organisms from oysters formed less acid from glucose than did similar organisms derived from man. He concludes: "The bacillus coli isolated from feces, both from laboratory assistants and from the immigrants of the *S. S. Roma,* produced more acid in dextrose and lactose broth than the colon bacillus isolated from oysters. This seems to indicate that Bacillus coli loses some of its ability to ferment carbohydrate with the production

of acid during the journey from the intestinal tract to the oysters." He states, however, that in his laboratory experiments he was unable to cause a reduction in fermenting power even after long periods (8 weeks) of storage in sea water.

It appears from this study that a very plausible explanation of Browne's results is that Voges-Proskauer-positive organisms were among his oyster strains. Such organisms are very rare in feces, but not uncommon in sewage and soil washings. The admixture of a



PLOT IV
Acid-Production by Voges-Proskauer Positive and Negative Coli-Like Bacteria

few Voges-Proskauer-positive organisms in a collection of colon-bacillus-like strains would decrease the mean amount of acid formed from glucose and raise the titer of that from sucrose. The oyster strains employed by Browne formed less acid from glucose, and somewhat more from sucrose, than the fecal strains, thus confirming to some extent the inference that the differences he observed were due to an admixture of a few Voges-Proskauer-positive organisms rather than to a loss of fermenting power by colon-bacillus-like strains in their passage through sewage.

THE SUBSTITUTION OF QUANTITATIVE ACID-PRODUCTION FOR GAS-FORMATION AS A DIFFERENTIAL INDEX IN STUDIES ON B. COLI

Kligler[6] suggests that quantitative acid-production be substituted for gas-formation as an index of fermentation. He points out that in standard meat-infusion sugar-freed carbohydrate broth media there is a rather sharp dividing line between acid-producers and nonacid-producers at 1.5% normal acid, and that quantitative gas-production is variable and unreliable. Of course it is agreed that, as a quantitative test, gas-formation as ordinarily determined in the Smith or Durham tube is of little value; as a qualitative test, however, it may be of considerable significance. If a culture is inoculated into sugar broth and gas is formed, while no gas is produced in plain broth, the organism would most certainly be regarded as a fermenter irrespective of whether more or less than 1.5% acid is formed.

Kligler apparently regards such an organism as a nonfermenter, for he says: "The members of the proteus group, on the other hand, produced from 10 to 20 per cent. gas in lactose broth tho at no time did they produce more than 1.0 percent normal acid," and he later records this group as lactose-negative. It is not the intention to debate at this point whether B. proteus is a lactose-fermenter or not, but it should be pointed out that to say that an organism which forms gas from a carbohydrate is a nonfermenter because the acid titer is low, introduces confusion into the already much maligned and abused term "fermentation." The low titer might be due to a secondary alkali-production which masks the acid, as suggested by Rogers. It has been repeatedly observed in this laboratory that B. aerogenes in peptone dipotassium-phosphate solution containing 1 or 2% glucose, may be acid to methyl red after 24 hours' incubation, but alkaline after from 48 to 96 hours at 37 C.

Rogers, Clark, and Evans[7] also determined titratable acid and selected 1% normal acid as the point of demarcation between fermenters and nonfermenters, but they point out the possible errors in acid-determination and give precedence to gas-formation as indicated in the following:

"Under certain circumstances which have not yet been definitely determined, the acid from the fermentation of sugar may be masked by a secondary alkaline production, sufficient in some cases entirely to obscure the acid for-

[6] Jour. Infect. Dis., 1914, 15, p. 137.

[7] (a) Jour. Infect. Dis., 1914, 14, p. 411; (b) 15, p. 100; (c) 1915, 17, p. 137.

mation. In one small group of this collection, the lactose broth tubes at the end of seven days were only slightly more acid than the blank, altho all of the cultures gave gas in lactose bile. In no case was the titration of the culture less than that of the blank, altho this was usually the case with broths in which there was no fermentation. Where positive evidence of the fermentation of a sugar was obtained in another way, the negative evidence of the titration was disregarded and in the correlation tables the culture was included with the positive reactions. If, for instance, the titration of lactose broth was negative, while the lactose bile fermentation tubes showed gas, the cultures were considered to be lactose positive."

Acid-production should not be given precedence over gas-formation. They may be independent characters. If however, after careful studies, it appears that there is a marked correlation between quantitative acid-production and qualitative gas-formation, then it may be feasible to supplement, if not substitute, the gas test by the acid test. In that event, the line of demarcation between fermenters and non-fermenters would have to be determined for the medium employed. In this study, with peptone water containing 1% carbohydrate, non-fermenters rarely produced as much as 0.2% normal acid.

Another point of disagreement as to acid-production by B. coli is the maximal amount of acid formed. Kligler,[6] using meat-infusion media, often obtained titers of 4% normal acid or more, and similar results have been recorded by Rogers.[7] Browne,[1] however, using Liebig's meat-extract media, states that the limiting acidity for B. coli is 2.4% normal acid as determined by titration with phenolphthalein. Winslow and Walker[8] determined the acid-production in 12 substances by B. coli. The maximal acidity observed was 0.45 c.c. N/20 NaOH to the cubic centimeter of culture medium, or 2.25% normal acid.

In the study recorded here, with peptone water as the basic medium, the results are in entire accord with Winslow and Walker's, and with Browne's. Of more than 2500 titrations, none showed more than 2.4% normal acid.

The difference in acid-production observed by various investigators is probably due to differences in the composition of the media employed. It is now well established that more acid is formed in meat-infusion broth than in beef-extract broth. In media containing much phosphates, as yeast water, even more acid is formed than in meat-infusion broth. Within certain limits the amount of acid formed, as determined by titration with phenolphthalein, is a function of the amount of buffer substances (as K_2HPO_4 amino-acids, extractives, etc.) present in the culture medium. Acid is formed until a certain

[8] Science, 1907, 26, p. 797.

H+-ion concentration is reached. The ratio of the total titratable acid formed to the maximal or limiting H+-ion concentration is not constant, but varies, within limits, with the amount of buffer materials present in the medium.

The limiting H+-ion concentration may be an index of (1) the resistance of an organism to acid (H+ ions), or (2) the point of equilibrium between the decomposing carbohydrate and its end products under the influence of an organism.

If the limiting H+-ion concentration in glucose broth is such as to inhibit further growth of the organism, then the organism will die and the H+-ion concentration will remain constant. This seems to be the course of events with the Voges-Proskauer-negative group. With the Voges-Proskauer-positive organisms the H+-ion concentration rises to a maximum and then decreases, the medium becoming alkaline to methyl red. Under these conditions it is inferred that the maximal H+-ion concentration is a measure of the point of equilibrium between glucose and its end products under the influence of the organism in question.

It may be further considered that after the limiting H+-ion concentration is reached, the organism, if not destroyed, will, if capable, attack the peptones forming alkali. Some of the free acid becomes neutralized and more carbohydrate may be decomposed. The H+-ion concentration would remain constant as long as there is any fermentable carbohydrate present. If this assumption is correct, then an increase of the carbohydrate should retard the reversion from an acid to an alkaline reaction. This is exactly what takes place. In some work now in progress it has been found that Voges-Proskauer-positive strains were alkaline to methyl red after 24 hours' incubation in 0.5% peptone dipotassium-phosphate solution containing 0.5% glucose. In the same medium with 1% glucose, the reaction was acid after 24 hours but alkaline after from 48 to 72 hours. With 2% glucose, the acid reaction persisted until the 4th or 5th day. With 5% glucose there was no reversion to an alkaline reaction even after several weeks.

THE CORRELATION OF ACID- AND GAS-FORMATION

Table 12 shows the relation of gas-production to the amount of acid formed from sucrose, raffinose, dulcitol, glycerol, and salicin. The other test substances are not indicated because they were invariably fermented with production of gas. Cultures are regarded as gas-

formers if gas is observed in the closed arm irrespective of the quantity.

Table 12 indicates that with sucrose, raffinose, and dulcitol, acid- and gas-production after 36 hours' incubation at 37 C. are strikingly correlated. Of 80 organisms which fail to form gas from sucrose, 79 (98.8%) form less than 0.2% normal acid, and the remaining culture forms only 0.2% normal acid. Among the 75 organisms which do give gas from sucrose, 8 (10.6%) form more than 0.4 but less than 0.6%, 48 (64%) give between 0.6 and 0.79%, and the remaining 19 strains (25.4%) form more than 0.8% normal acid. There is no overlapping whatever between the amounts of acid produced by the gas-formers and the nongas-formers. To summarize: of the 80 strains which fail to produce gas, none form more than 0.2% normal acid, while among the 74 gas-producers the minimal amount of acid produced in peptone solution containing 1% sucrose is more than 0.4% normal acid.

A similar correlation is observed between acid- and gas-formation in 1% dulcitol in peptone solution. Of 88 strains that do not form gas, 86 (97.8%) give less than 0.2% normal acid. The remaining two organisms form 0.3% and 0.4% normal acid. Among the 67 gas-formers, however, there are only 2 (3%) that produce less than 0.4% normal acid.

The correlation of acid- and gas-production in peptone raffinose solution is also very marked; 79 produce gas and 77 fail to form gas from raffinose. Of the nongas-formers 72 (93.5%) form less than 0.2% normal acid; 3 organisms (3.9%) between 0.2% and 0.6% acid; and 2 cultures (2.6%) more than 0.8% acid. Among the gas formers 1 culture (1.3%) produces no acid, while 2 others (2.5%) form less than 0.6% normal acid. The other 76 gas-formers (96.2%) form more than 0.6% normal acid.

With glycerol and salicin the correlation of acid-production and gas-formation is not nearly so striking as it is with sucrose, dulcitol, or raffinose.

Gas is formed from glycerol by 118 of the cultures after 72 hours' incubation, while 38 organisms do not form gas. Of the gas-formers, 16 (13.6%) produce 0.4-0.59% normal acid as compared with 23 (60.6%) of the nongas-formers, while 61 (51.7%) of the former and 5 (13.2%) of the latter give 0.6-0.79% normal acid. One organism which does not form gas yields more than 0.8% normal acid.

TABLE 12

RELATIONSHIP BETWEEN QUANTITATIVE ACID-PRODUCTION AND GAS-FORMATION BY B. COLI

Test Substance	Gas		Percentage or Normal Acid				
			0-0.19	0.20-0.39	0.40-0.59	0.60-0.79	0.80 or more
Sucrose	+	No.	0	0	8	48	19
		%			10.6	64.0	25.4
	−	No.	79	1	0	0	0
		%	98.8	1.2			
Raffinose	+	No.	1	0	2	18	58
		%	1.3		2.5	22.8	73.4
	−	No.	72	1	2	0	2
		%	93.5	1.3	2.6		2.6
Dulcitol	+	No.	0	2	5	23	37
		%		3.0	7.5	34.3	55.2
	−	No.	86	1	1	0	0
		%	97.8	1.1	1.1		
Salicin	+	No.	0	0	1	19	82
		%			10	18.6	80.4
	−	No.	43	1	3	6	1
		%	79.7	1.8	5.6	11.1	1.8
Glycerol	+	No.	0	0	16	61	41
		%			13.6	51.7	34.7
	−	No.	4	5	23	5	1
		%	10.5	13.2	60.5	13.2	2.6

Salicin is fermented, with gas-formation, by 102 organisms after 72 hours at 37 C., while 54 strains do not form gas. Among the non-gas-formers, 10 (18.5%) produce 0.4-0.8% normal acid, whereas this quantity of acid is also formed by 20 (19.6%) of the gas-formers.

It appears from Table 12 that under the conditions of these experiments, acid-production in sucrose, dulcitol, and raffinose is well correlated with the presence or absence of gas. With salicin the correlation is not so marked, while with glycerol the line of demarcation between gas-formers and nongas-formers, as indicated by the quantity of acid produced, is very indistinct. The substitution of quantitative acid-production for gas-formation would therefore be particularly undesirable when working with glycerol.

These results are well in accord with those of Winslow and Walker,[8] who observe: "Gas-formation coincided with acidity except in the case of dextrin." Unfortunately, acid-formation in dextrin was not determined in this study, and Winslow and Walker did not employ salicin or glycerol.

CHARACTERISTICS OF ORGANISMS FROM THE DIFFERENT SOURCES

When this study was begun (1915), motility and fermentation of dextrin and starch were regarded as of little significance and hence these tests were omitted. In the following year (1916) the possible significance of the reactions was realized and, as the cultures were still available, they were tested out. Motility was determined in a soft agar medium consisting of nutrient broth and 0.5% agar.

In Table 13 are shown the number and percentage of organisms giving positive reactions with the various tests. Glucose, galactose, mannitol, maltose, and lactose are fermented by all strains, with gas-production. Inulin is not fermented by any of the organisms, and gelatin is uniformly negative in 20 days at 20 C. Gas is formed from glycerol by 76.2%, from salicin by 66.1%, from raffinose by 50.7%, from sucrose by 48.7%, from dulcitol by 43.6%, from dextrin by 5.1%, and from starch by 4.5%. The Voges-Proskauer reaction is given by 5.8%, indol is produced by 91.1%, and 61.5% are motile.

Table 14 shows the characters of organisms isolated from different sources. Characters which are negative or positive for all strains are omitted.

Several things are evident. Organisms giving a positive Voges-Proskauer reaction or gas from dextrin and starch were obtained

TABLE 13

GAS-FORMATION AND OTHER CHARACTERISTICS OF BACILLUS-COLI-LIKE BACTERIA FROM VARIOUS ANIMALS AND SEWAGE

Character	Number Positive	Percentage Positive
Motility	96	61.5
Gelatin	0	0
Indol	142	91.1
Voges-Proskauer	9	5.8
Glucose	156	100
Galactose	156	100
Mannitol	156	100
Dulcitol	68	43.6
Glycerol	118	76.2
Maltose	156	100
Lactose	156	100
Sucrose	76	48.7
Raffinose	79	50 7
Salicin	102	66.1
Dextrin	8	5.1
Inulin	0	0
Starch	7	4.5

only from sewage. This must not be taken to mean that such organisms are entirely absent from the other sources, but it certainly indicates that they are extremely scarce in feces of the animals studied.

Salicin is fermented by 95% of the bovine strains, and by 8 (89%) of the 9 Voges-Proskauer-positive strains from sewage. Organisms

TABLE 14

MOTILITY AND OTHER REACTIONS OF BACILLUS-COLI-LIKE BACTERIA FROM DIFFERENT SOURCES

Source	Horse	Sheep	Cow	Pig	Man	Sewage	
						V. P. —	V. P. +
Number of strains	19	22	20	31	25	30	9
	Percentage of Positive Reactions						
Motility	100.0	77.3	80.0	93.7	32.0	20.0	11.1
Voges-Proskauer reaction	0.0	0.0	0.0	0.0	0.0	0.0	100.0
Indol	100.0	100.0	100.0	93.7	84.0	83.5	66.7
Sucrose	79.0	95.5	50.0	32.3	12.0	26.6	100.0
Raffinose	73.8	100.0	50.0	32.3	16.0	33.3	100.0
Dulcitol	68.5	50.0	50.0	42.0	20.0	43.3	33.3
Glycerol	84.3	62.0	95.0	74.2	64.0	70.0	88.9
Salicin	73.8	68.3	95.0	58.1	44.0	60.0	88.9
Dextrin	0.0	0.0	0.0	0.0	0.0	0.0	88.9
Starch	0.0	0.0	0.0	0.0	0.0	0.0	77.8

from other sources attack salicin less readily—horse 73.8%, sheep 68.3%, pig 58.1%, man 44%, and sewage (Voges-Proskauer-negative strains) 56.7%. This glucosid was used by MacConkey,[3] who did not regard its employment worth while for classification purposes. Recently (1914) Kligler[6] suggested that salicin displace dulcitol in

subdivision of the colon-bacillus group, but Rogers[7] questions the value of salicin in view of the very large number of his strains which attacked it. In this connection it might be well to point out that organisms studied by Rogers consisted of bovine strains, grain strains (probably Voges-Proskauer-positive organisms), and milk strains (which may be considered for the most part as a mixture of bovine and grain strains). In view of the results obtained here with bovine and Voges-Proskauer-positive strains, and by Kligler with Voges-Proskauer-positive strains, it would be expected that more than 90% of Rogers' cultures would attack salicin. It appears then that salicin-fermentation is somewhat correlated with the source.

Glycerol is also fermented by almost all the Voges-Proskauer-positive and bovine strains and less frequently by organisms from the other animals, but the difference is less marked than with salicin.

Dulcitol is only occasionally fermented by the human and Voges-Proskauer-positive strains, but there seems to be very little relation between dulcitol-fermentation and the animal source.

Indol-production is not correlated with the animal source.

In motility there is a marked contrast between the strains from horse, sheep, cow, and pig on the one hand, and those from man and sewage on the other. Less than one-third of the sewage and human strains are motile, as compared with more than four-fifths of the other animal strains. McWeeney[9] found nonmotile B. coli abundant in feces, and notes that Stocklin also had observed many nonmotile forms among fecal strains. Just what significance is to be attached to motility is hard to say at present, because so few bacteriologists determine this character in routine work. MacConkey, however, strongly advocates the test. As determined in the 0.5% agar medium the motility test is simple, quick, and not at all burdensome.

Sucrose and raffinose are so well correlated that a consideration of either will suffice for both. The Voges-Proskauer-positive and sheep strains are practically all sucrose-fermenters (100% and 95.5% respectively). Of the horse strains 79%, and of the organisms from the cow 50% form gas from sucrose; only 32.3% of strains from the pig, 26.6% of those from sewage (Voges-Proskauer-negative strains), and 12% of those from man form gas from sucrose. That such a small number of human strains attack sucrose is particularly interesting, and a review of the literature indicates that similar results have

[9] Cited by Prescott and Winslow, Elements of Water Bacteriology, 1913.

TABLE 15

FERMENTATION OF SUCROSE BY BACILLUS-COLI-LIKE BACTERIA FROM HUMAN FECES

Investigators	Number of Organisms Studied	Number of Sucrose Fermenters	Percentage of Sucrose Fermenters
Houston,[11] 1902-3	100	30	30
MacConkey,[3] 1905 and 1909	419	142	33.9
Ferreira,[12] Horta, Paredes, 1908	117	44	37.6
Winslow[8] and Walker, 1907	25	8	32
Howe,[10] 1912	540	324	60
Clemesha,[13] 1912	1200	348	29
Browne,[1] 1915	175	20	11.3
Levine,[4] 1916	25	3	12
Total	2601	919	35.3

been obtained by previous investigators. In Table 15 is shown the proportion of sucrose-fermenters obtained from human feces by different investigators. Howe[10] found 60% of 540 Bacillus-coli-like organisms to be sucrose fermenters, but the other investigators usually found twice as many nonfermenters as fermenters. Of 2601 cultures of human colon bacilli studied by various observers, at different times and in different countries, only 35.3% fermented sucrose.

In connection with the study reported here it should be noted that the number of human strains isolated is small, and that they were collected in the winter. Clemesha,[13] and also Browne,[1] call attention to "epidemics" of certain types of B. coli, and to seasonal variations. These phases need further investigation.

CONCLUSIONS

In studies on quantitative acid-production the average should be supplemented with a statement of its deviation measures; the unqualified average may lead to a misconception of the acid-producing properties of a group of organisms.

Quantitative acid-production in glucose, galactose, maltose, lactose, sucrose, raffinose, salicin, inulin, mannitol, dulcitol, and glycerol, is not a reliable index for differentiating colon-bacillus-like bacteria derived from pig, horse, sheep, cow, or man.

The MacConkey types are indistinguishable on the basis of quantitative acid-production in the fermentable carbohydrates, the alcohols, and the glucosid studied.

[10] Science, 1912, 35, p. 225.
[11] Suppl. to 32nd Ann. Rep. containing Rep. of Med. Officer for 1902-1903, p. 511.
[12] Arch. d. Real Inst. Bacteriol. Camara Pestana, 1908, 2, p. 153.
[13] Jour. Hyg., 1912, 12, p. 463.

The Voges-Proskauer-positive strains (aerogenes-cloacae group) form somewhat less acid from glucose, but more acid from maltose, sucrose, glycerol, and dulcitol, and possibly also from raffinose and salicin, than do the Voges-Proskauer-negative strains (colon-bacillus group).

Acid-formation should not be given precedence over gas-formation in studies on B. coli, for the acid may be masked by a secondary alkali-production. In general, however, acid-production is accompanied by gas-formation. With sucrose, dulcitol, and raffinose, acid-production and gas-formation are almost perfectly correlated. The correlation is less marked in the case of salicin, while the line of demarcation between gas-formers and nongas-formers, as indicated by quantitative acid-production from glycerol, is very indistinct.

Practically all Voges-Proskauer-positive and bovine strains attack salicin with liberation of gas. This glucosid is fermented less frequently by the organisms from pig, horse, sheep, man, and sewage

Gas is formed from sucrose as follows: Voges-Proskauer-positive (Voges-Proskauer-negative strains).
strains 100%, sheep 95.6%, horse 79%, cow 50%, pig 32.3%, sewage (Voges-Proskauer-negative strains) 26%, and human strains 12%.

Of 2601 human strains of B. coli studied by different investigators, in various countries and at different times, only 35.3% have been sucrose-fermenters.

Motility, as determined in semisolid nutrient agar, seems to be an important character. Only 32% of the human and 20% of the Voges-Proskauer-negative sewage strains are motile, as compared with 93.7% of pig, 80% of cow, 77.3% of sheep, and 100% of horse strains.

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A STATISTICAL CLASSIFICATION OF THE COLON-CLOACAE GROUP

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It is now firmly established that the end products of metabolism, as well as morphological differences, are reliable and convenient indices for differentiation of bacterial species and varieties. In the group of coli-like bacteria, particular attention has been paid to acid and gas production fron various fermentable substances.

Theobald Smith (1893) observed that some *B. coli* ferment sucrose and therefore recognized two forms.

Durham (1901) suggested the name *B. coli-communior* for the sucrose fermenting variety and characterized *B. lactis-aerogenes* as a polysaccharid fractor.

MacConkey (1905 and 1909), whose classification has been most widely employed, subdivided upon gas formation from sucrose and then from dulcitol thus giving 4 main types generally known as the *B. acidi-lactici* type (sucrose negative, dulcitol negative); the *B. communis* type (sucrose negative dulcitol positive); the *B. communior* type (sucrose positive, dulcitol positive), and the *B. aerogenes* type (sucrose positive, dulcitol negative). Under each type are recorded a number of varieties according to gelatin liquefaction, indol production, the Voges-Proskauer reaction, motility, and fermentation of inulin, adonitol, etc.

Very much along the lines of the MacConkey classification is that of Bergey and Deehan (1908). They employed 8 characters —fermentation of sucrose, dulcitol, adonitol, and inulin; gelatin liquefaction, indol production, motility and Voges-Proskauer reaction—and from a consideration of all possible combinations

between these characters recognized the possible existence of 256 varieties of *B. coli.*

The grouping of Jackson (1911) which was accepted by the American Public Health Association and included in the standard methods for 1912, is very similar to that of MacConkey, but here preference is given to dulcitol over sucrose for the primary division. Each of the 4 groups thus formed is subdivided further on raffinose and mannitol, and then on motility, indol, reduction of nitrates, and gelatin liquefaction.

A very serious objection to the classifications of MacConkey, Bergey and Deehan, and Jackson, is their extreme flexibility. As the number of fermentable substances, or other characters observed, increases, the number of "varieties" increases geometrically approaching infinity. The number of "varieties" is given by the formula 2^n where 'n' is the number of characters studied. Thus with 8 characters there are 256 possible combinations; this number rises to 1024 with 10 characters and to 65,536 when 16 characters are observed. The absurdity of regarding each character as of similar and equal differential value is thus evident. In the more recent studies the principle of the correlation of characters has been emphasized.

Howe (1912), from a statistical study of 630 strains of *B. coli* isolated from human feces, concludes that dulcitol, indol production, nitrate reduction, etc., are not correlated with each other nor with vigor of growth, and he therefore recognizes only the sucrose positive *B. communior* and sucrose negative *B. communis.*

Rogers and his associates, (1914–1916) studied a large number of coli-like forms from milk, grains, and bovine feces, and conclude that two distinct groups may be recognized on the basis of the accurately determined gas ratio—the low ratio *B. communis-B. communior* group and the high ratio *B. aerogenes-B. acidi-lactici* group. There is no doubt that *B. communis* and *B. communior* are low ratio strains and *B. aerogenes* of the high ratio group but the inclusion of *B. acidi-lactici* with *B. aerogenes* does not seem justified, and I believe that further studies will place it definitely with the low ratio strains.

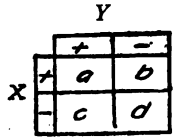
Kligler (1915) suggests that salicin be substituted for dulcitol, in subdividing coli-like bacteria, pointing out that salicin fermentation correlates better with the Voges-Proskauer reaction than does dulcitol decomposition. He thus recognizes a sucrose negative, salicin negative group (*B. acid-lactici*); sucrose negative, salicin positive group (*B. communis*); sucrose positive, salicin negative group (*B. communior*) and sucrose positive, salicin positive (*B. aerogenes*). *B. cloacae* is differentiated from *B. aerogenes* by its inability to ferment glycerol.

The characterization of *B. communior* as salicin negative is probably untenable. The term *B. coli-communior* was first employed by Durham to describe a variety of *B. coli* which fermented sucrose and which was motile. Later Ford recognized it as a species *B. communior*. Such organisms usually ferment salicin as will be shown later in this paper.

Where the principle of correlation has been employed the best correlated character has apparently been picked out by inspection of the data. Inspection is a tedious and difficult procedure, entirely inapplicable where the number of characters considered is large, and it does not permit of a concise statement of the degree of correlation which exists between different reactions. Considerable information in an abstract, concise, and workable, form may however be obtained from a study of the coefficients of correlation.

THE COEFFICIENT OF CORRELATION

Where we are concerned merely with the presence or absence of characters the coefficient of correlation between any two characters may be easily determined. Suppose that it is desired to know if the characters X and Y are correlated and that a study of a number of organisms showed that 'a' cultures are positive for both X and Y; 'b' organisms positive for X but negative for Y, 'c' cultures are negative for X and positive for Y; and 'd' strains are negative for both X and Y. The distribution of the organisms is first tabulated as shown below.



The degree of association, or the coefficient of correlation, is then expressed, according to Yule, by the formula

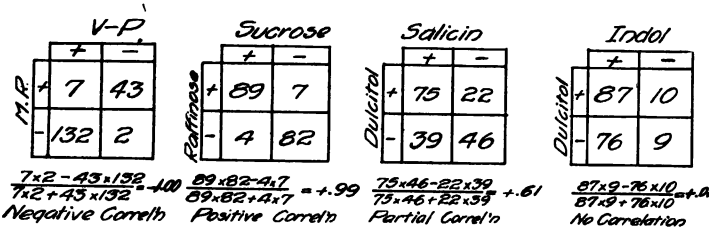
$$\frac{ad - bc}{ad + bc} \qquad (1)$$

If 'ad' is equal to 'bc' the coefficient becomes $\frac{0}{ad + bc}$ or 0; which indicates that there is no correlation whatever. If either 'b' or 'c' is zero the formula becomes $\frac{ad}{ad} = 1$; indicating a perfect positive correlation. If 'a' or 'd' is zero then we have $\frac{-bc}{bc} = -1$; showing a perfect negative correlation. It should be observed that an absolute positive correlation exists in reality only if both 'b' and 'c' are zero and an absolute negative correlation when both 'a' and 'd' are zero. In order to avoid coefficients of 1 or -1 where only one group—'a', 'b,' 'c,' or 'd'—is zero. Yule gives the formula

$$\frac{a\,(a+b+c+d) - (a+c)\,(a+b)}{\sqrt{(a+c)\,(b+d)\,(a+b)\,(c+d)}} \qquad (2)$$

In practice, however, a few strains are almost always found in each of the four groups and Yule suggests the use of the simpler formula (1). Some caution should therefore be employed in interpreting coefficients of 1 or -1.

For this study it was assumed that if the coefficient between two characters is numerically greater than 0.5 they may be regarded as correlated, but if less than 0.3 there is probably no association. A few examples of correlation coefficients actually obtained in the course of this study are given to illustrate the method of calculation.



The principle of correlation should not be applied indiscriminately to collections of data for systematic purposes. Certain characters and properties have been universally accepted as reliable and appropriate for bacterial differentiation; thus, staining reactions such as the Gram and acid fast stains; spore formation, aerobiosis and anaerobiosis, hardly need to be bolstered up by correlation with other characters to justify their taxonomic value. On the other hand the significance of such characters as motility, indol production, and fermentation of certain substances, is still debatable.

Motility is regarded by many as a highly variable property. Perhaps it is in reality a reliable morphological difference. Certainly if it could be shown that this character goes hand in hand with several others, more reliance and attention should and would be given to motility. The same is true of the indol test. In dealing with gas formation from carbohydrates, alcohols, or polysaccharids, the question naturally arises as to which substance should be given preference for subdivision, or whether all are to be considered of equal taxonomic value. The lack of a criterion for determining the most significant fermentable substances has led to considerable confusion. It has already been pointed out how subdivision on every character studied results in an infinite number of varieties. Where we are dealing with a number of characters each of which is assumed to be of equal taxonomic significance, it would certainly be desirable and advantageous to subdivide on that character which gives the greatest amount of information as to the manner in which the resulting subgroups react with respect to other characters. It is under such circumstances that the principle of correlation of characters may be legitimately, conveniently, and advantage-

ously employed. It may be recalled that the differentiation of the colon-intermediate-typhoid group on glucose and lactose fermentation is strikingly correlated with pathogenicity.

STATISTICAL STUDY

In the following pages is evolved a classification of coli-like bacteria based primarily upon correlated characters. The study is made upon 333 organisms obtained from soil, sewage and the feces of man, horse, sheep, pig and cow.

The characters considered are the methyl-red and Voges-Proskauer reactions, indol production, motility, gelatin liquefaction and gas formation from sucrose, raffinose, dulcitol, glycerol, salicin, dextrin, inulin and corn starch. Other fermentable substances—lactose, maltose, galactose and mannitol—were also observed but as these substances were all attacked with gas formation they need not be considered.

The investigations of Theobald Smith, Hardin, Rogers and others indicate distinctly that the Voges-Proskauer positive or methyl-red negative strains are so different from the Voges-Proskauer negative, or methyl-red positive organisms with respect to the end products of carbohydrate fermentation that subdivision upon these characters seems justified. Two groups are therefore recognized, the methyl-red positive, Voges-Proskauer negative or *B. coli group* and the methyl-red negative, Voges-Proskauer positive or *B. aerogenes-B. cloacae* group.

METHOD OF STUDY

The organisms in each of the two groups are first tabulated as in table 1 in order to facilitate the calculation of the correlation coefficients which are then determined for each pair of characters and recorded as indicated in table 1A. In choosing between any two characters, that one which gives the highest coefficient of correlation with the greatest number of other characters is selected for subdivision. For the resulting subgroups new correlation tables are prepared and subdivision again made as above. A point is very quickly reached where further subdivision upon

correlated characters is no longer feasible. These groups are regarded as species and to each is assigned, as far as possible, the name of the MacConkey variety which it most resembles.

TABLE 1

Showing correlation of characters among 151 strains of the Aerogenes-cloacae group

		Gelatin		Motility		Indol		Sucrose		Raffinose		Dulcitol		Glycerol		Salicin		Dextrin		Inulin*		Starch	
		+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Gelatin	+	83		81	2	13	70	83		79	4	11	72	7	76	82	1	27	56	4	79	5	78
	−		68	8	60	33	35	65	3	66	2	34	34	62	6	67	1	63	5	18	41	60	8
Motility	+	81	8	89		15	74	86	3	83	6	14	75	8	81	87	2	28	61	4	84	4	85
	−	2	60		62	31	31	62		62		31	31	61	1	62		62		18	36	61	1
Indol	+	13	33	15	31	46		46		46		24	22	37	9	46		39	7	9	31	35	13
	−	70	39	74	31		103	102	3	99	6	21	84	32	73	103	2	51	54	13	89	32	73
Sucrose	+	83	65	86	62	46	102	148		144	4	44	104	69	79	146	2	90	58	22	117	65	83
	−		3	3			3		3	1	2	1	2		3	3			3		3		3
Raffinose	+	79	66	83	62	46	99	144	1	145		44	101	68	77	143	2	88	57	21	115	65	80
	−	4	2	6			6	4	2		6	1	5	1	5	6		2	4	1	5		6
Dulcitol	+	11	34	14	31	24	21	44	1	44	1	45		38	7	45		37	8	11	27	33	12
	−	72	34	75	31	22	84	104	2	101	5		106	31	75	104	2	53	53	11	89	32	74
Glycerol	+	7	62	8	61	37	32	69		68	1	38	31	69		69		66	3	20	41	63	6
	−	76	6	81	1	9	73	79	3	77	5	7	75		82	80	2	24	58	2	79	2	80
Salicin	+	82	67	87	62	46	103	146	3	145	6	45	104	69	80	149		89	60	22	119	65	84
	−	1	1	2			2	2		2			2		2		2	1	1		1		2
Dextrin	+	27	63	28	62	39	51	90		88	2	37	53	66	24	89	1	90		22	60	65	25
	−	56	5	61		7	54	58	3	57	4	8	53	3	58	60	1		61		60		61
Inulin*	+	4	18	4	18	9	13	22		21	1	10	11	20	2	22		22		22		21	1
	−	79	41	84	36	31	89	117	3	115	5	22	89	41	79	119	1	60	60		120	36	84
Starch	+	5	60	4	61	33	32	65		65		33	32	63	2	65		65		21	36	65	
	−	78	8	85	1	13	73	83	3	80	6	12	74	6	80	84	2	25	61	1	84		86

* Nine strains not tested in inulin.

THE AEROGENES-CLOACAE GROUP

In the *B. aerogenes-B. cloacae* group are included all strains which gave the Voges-Proskauer reaction—(practically always alkaline to methyl-red) and 10 cultures which fermented starch with gas formation but did not react typically for the Voges-Proskauer nor methyl-red tests. There are 151 organisms in the group, 9 of which were obtained from sewage and the rest, 142, from soil.

The distributions of the strains with respect to gelatin liquefaction, motility, indol and gas formation from sucrose, raffinose, dulcitol, glycerol, salicin, dextrin, inulin and starch are shown in table 1. Mannitol, maltose, lactose and galactose were always fermented and are therefore not included.

Gelatin was liquefied by 83 (55 per cent) (observed for thirty-four days at 20°C.); 89 (59 per cent) were motile; 46 (30.5 per

TABLE 1A

Coefficients of correlation for each pair of characters in table 1

	Gelatin	Motility	Indol	Dulcitol	Glycerol	Dextrin	Inulin	Starch
Gelatin		+.99	-.67	-.74	-.98	-.93	-.79	-.98
Motility	+.99		-.66	-.69	-.99	-1.00	-.83	-1.00
Indol	-67	-.66		+.63	+.80	+.71	+.33	+.71
Dulcitol	-.74	-.69	+.63		+.86	+.65	+.62	+.73
Glycerol	-.98	-.99	+.80	+.86		+.97	+.90	+.99
Dextrin	-.93	-1.00	+.71	+.65	+.97		+1.00	+1.00
Inulin	-.79	-.83	+.33	+.62	+.90	+1.00		+.96
Starch	-.98	-1.00	+.71	+.73	+.99	+1.00	+.96	

cent) formed indol from Witte's peptone, and gas was formed as follows: sucrose 148 (98.2 per cent); raffinose 145 (96.2 per cent); dulcitol 45 (29.8 per cent); glycerol 69 (45.6 per cent); salicin 149 (98.8 per cent); dextrin 90 (59.5 per cent); inulin 22 (14.6 per cent) and starch 65 (43 per cent). It is evident from table 1 that sucrose, raffinose and salicin, because of their extreme availability, cannot be employed for differentiation within the group. The coefficients of correlation for each pair of remaining characters are given in table 1A.

Mere inspection of table 1A shows that gelatin liquefaction is almost perfectly correlated with motility and fermentation of glycerol, dextrin, and starch; the association being positive with motility and negative with the others. Similarly motility is

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correlated with glycerol, dextrin, starch and gelatin. Each of these characters is correlated with each other. Under these circumstances any of these reactions may be selected for subdivision; the choice depending upon which were employed in an investigation and to some extent on the personal preference of the investigator. The characterization of *B. aerogenes* by Durham as a starch fermenter; the differentiation of *B. aerogenes* from *B. cloacae* by MacConkey on gelatin liquefaction and motility, and by Kligler on glycerol fermentation are all correct; the apparent confusion being the inevitable result of separation upon single characters.

Two species are evidently present, the *B. aerogenes* which rarely, if ever, liquefies gelatin; is non-motile; and forms gas from glycerol and starch; and the *B. cloacae* which liquefies gelatin (often very slowly); is motile; and does not form gas from glycerol nor starch. As gelatin liquefaction is an inconvenient character the organisms are subdivided for further study upon motility into the non-motile *B. aerogenes* and the motile *B. cloacae*. Glycerol or starch would do just as well. Whichever character is selected, a few strains are present in each of the resulting groups which possess some of the salient characteristics of the other. Thus of 89 motile strains 8 did not liquefy gelatin, 8 formed gas from glycerol and 4 from starch, while of 62 non-motile strains, 2 liquefied gelatin, and glycerol and starch were attacked by one.

The presence of a few supposedly non-liquefiers among the motile strains may as probably—and even more probably—be an indication of the inaccuracy and unreliability of the gelatin liquefaction test than of the presence of true intermediate organisms, for the number of gelatin liquefiers recognized increases with the period of incubation. Again is it not reasonable to explain the presence of several glycerol and starch fermenters among the motile strains as due to mixed cultures? Picking off a colony from a plate, even after several replatings, is no absolute criterion that a pure culture was obtained. Some species stick tenaciously together.

One of the motile starch fermenting strains referred to above

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was plated out on brilliant green agar. Ten colonies were fished into motility agar and starch; three were non-motile starch fermenters, three were motile and did not attack starch, while four were both motile and starch fermenters thus indicating that, in this instance at least, the presumably overlapping or intermediate

TABLE 2

Showing correlation of indol, dulcitol, and inulin for 62 strains of B. aerogenes

		Indol		Dulcitol		Inulin *	
		+	−	+	−	+	−
Indol	+	31		20	11	7	18
	−		31	14	17	11	18
Dulcitol	+	20	14	34		8	20
	−	11	17		28	10	16
Inulin*	+	7	11	8	10	18	
	−	18	18	20	16		36

* Eight cultures not tested in inulin.

TABLE 2A

Coefficient of correlation for each pair of characters in table 2

	Indol	Dulcitol	Inulin
Indol		+.39	-.22
Dulcitol	+.39		-.22
Inulin	-.22	-.22	

strains are in all probability merely mixed cultures. It is not contended that intermediate strains do not occur; they undoubtedly do; but it is desired to point out that these have been over emphasized in the past and that the plating method cannot always be relied upon to yield pure cultures.

B. cloacae may be defined as a gram negative short rod which ferments lactose weakly; forms acetylmethylcarbinol from glucose; is alkaline to methyl-red; motile; rarely forms indol; prac-

tically always forms gas from sucrose, raffinose, mannitol and salicin; and occasionally from dextrin; gelatin is typically liquefied; and glycerol, inulin and starch are not fermented. As noted above, the few glycerol, inulin and starch fermenters are probably due to mixed cultures and may be dismissed.

The three sucrose negative cultures (also raffinose negative) may be regarded as a variety corresponding to the *B. levans* which MacConkey records as very rare.

The dextrin fermenters probably also constitute a variety of *B. cloacae* but as the composition of dextrin is so variable we hesitate to employ it for differential purposes for the present.

B. aerogenes resembles *B. cloacae* in several respects. It forms acetylmethylcarbinol from glucose; is alkaline to methyl-red; and ferments sucrose, raffinose, mannitol, and salicin with gas formation. On the other hand lactose is more vigorously attacked; gelatin is typically not liquefied; the organisms are non-motile; while glycerol and starch are fermented with gas formation.

Indol was formed by 31 (50 per cent); gas from dulcitol was formed by 31 (50 per cent); and from inulin by 18 (33⅓ per cent) of the *B. aerogenes* strains (8 cultures were not tested with inulin). From tables 2 and 2A which show the distribution with respect to indol, inulin and dulcitol, and the correlation coefficients for these reactions, it is evident that the characters are not associated. They may be of significance for separation of varieties. The utter lack of correlation necessitates the employment of all of these characters, which would lead to the formation of eight varieties. It is deemed unwise to establish such varieties until more extensive collections are studied.

THE COLI GROUP

In the *B. coli* group are included 182 strains quite evenly distributed between the different animal sources, sewage and soil. The group differs sharply from the *B. aerogenes-B. cloacae* series in that the Voges-Proskauer reaction is negative and the methyl-red reaction positive. Starch is not attacked by any of the strains. It has been shown by the author that the Voges-Pros-

kauer negative strains attack the monosaccharids more vigorously, but the disaccharids, trisaccharid, and glucoside no less vigorously than the Voges-Proskauer positive strains.

In table 3 is shown the correlation between the various reactions. As all strains attacked galactose, lactose, maltose and

TABLE 3

Showing correlation of characters among 182 strains of the coli group

		Motility		Indol		Sucrose		Raffinose		Dulcitol		Glycerol		Salicin	
		+	−	+	−	+	−	+	−	+	−	+	−	+	−
Motility	+	130		114	16	77	53	77	53	80	50	92	37	89	41
	−		52	49	3	16	36	19	33	17	35	33	19	25	27
Indol	+	114	49	163		84	79	86	77	87	76	110	52	109	54
	−	16	3		19	9	10	10	9	10	9	15	4	5	14
Sucrose	+	77	16	84	9	93		89	4	64	29	56	36	63	30
	−	53	36	79	10		89	7	82	33	56	69	20	51	38
Raffinose	+	77	19	86	10	89	7	96		65	31	60	35	66	30
	−	53	33	77	9	4	82		86	32	54	65	21	48	38
Dulcitol	+	80	17	87	10	64	33	65	32	97		63	34	75	22
	−	50	35	76	9	29	56	31	54		85	62	22	39	46
Glycerol	+	92	33	110	15	56	69	60	65	63	62	125		89	36
	−	37	19	52	4	36	20	35	21	34	22		56	25	32
Salicin	+	89	25	109	5	63	51	66	48	75	39	89	25	114	
	−	41	27	54	14	30	38	30	38	22	46	36	32		68

mannitol with gas formation and failed to attack the polysaccharids, dextrin, inulin and starch, or to liquefy gelatin, these substances are not included in the table.

The proportion of positive reactions for all strains of the *B. coli* group is as follows: motility 130 (71.5 per cent); indol 163 (89.6 per cent); sucrose 93 (51.1 per cent); raffinose 96 (52.7 per cent); dulcitol 97 (53.3 per cent); glycerol 125 (68.8 per cent) and salicin 114 (62.7 per cent).

The correlation coefficients for each pair of characters is given in table 3A.

The highest coefficient obtained for motility is 0.53 with both

sucrose and dulcitol. Its correlation with other characters is therefore not very marked.

Indol seems to be correlated with salicin, but the small proportion of indol negative strains (10.4 per cent) makes the association of questionable value, and as the coefficients with other substances are extremely low, indol may be eliminated.

Glycerol correlates somewhat with salicin (coefficient 0.52) but

TABLE 3A

Coefficients of correlation for each pair of characters in table 3

	Motility	Indol	Sucrose	Raffinose	Dulcitol	Glycerol	Salicin
Motility		-.39	+.53	+.43	+.53	+.18	+.40
Indol	-.39		+.08	+.00	+.02	-.28	+.76
Sucrose	+.53	+.08		+.99	+.58	-.38	+.20
Raffinose	+.43	+.00	+.99		+.58	-.29	+.27
Dulcitol	+.53	+.02	+.58	+.58		-.21	+.60
Glycerol	+.18	-.28	-.38	-.29	-.21		+.52
Salicin	+.40	+.76	+.20	+.27	+.60	+.52	

with no other character and is therefore not considered desirable for subdivision at this point.

The choice of a differential character is thus narrowed down to sucrose, raffinose, dulcitol and salicin. Sucrose and raffinose are almost perfectly associated (coefficient of correlation 0.99). Consideration of either therefore suffices for both and as the former is slightly better correlated with other characters, sucrose is selected for further discussion.

A comparison of salicin with dulcitol indicates that the alcohol is to be preferred. Salicin correlates better with glycerol and indol (the latter relation of questionable value), while dulcitol has higher coefficients with motility, sucrose and raffinose. A similar consideration leads to the choice of sucrose over salicin.

Sucrose and dulcitol therefore remain. These are the two characters in regard to which there is considerable difference of opinion among students of the *B. coli* group. MacConkey gives preference to sucrose, and in this selection is supported by many investigators (Howe 1912, Kligler 1915, Rogers 1915, etc.); while Jackson (1911) and more recently Giltner (1916) subdivide first on dulcitol. It is quite interesting, therefore, that on the basis of the correlation coefficients there is really little choice between the two. Both are equally well correlated with motility (coefficient 0.53); partially with each other (coefficient 0.58) and not associated with indol. Dulcitol correlates partially with salicin (coefficient 0.60), while sucrose does not (coefficient 0.20). On the other hand, sucrose is almost perfectly correlated with raffinose (coefficient 0.99), whereas salicin is only partially (coefficient 0.58). Although neither can be regarded as associated with glycerol, the coefficient with sucrose (-0.38) is greater than with dulcitol (-0.21).

If our selection is to be guided entirely by correlation, the choice between dulcitol and sucrose is a toss up. Sucrose was finally selected for the primary division because it is more widely distributed in nature, more available to students for investigational purposes, more widely accepted by bacteriologists, and its fermentation better correlated with the source than is dulcitol decomposition. Differentiation on sucrose yields a sucrose positive group of 93 strains, and a sucrose negative group of 89 strains.

THE SUCROSE NEGATIVE STRAINS OF THE COLI GROUP

Of the 89 strains which did not form gas from sucrose, 33 (37.1 per cent) were positive in dulcitol; 69 (77.6 per cent) positive in glycerol; and 51 (57.3 per cent) gave gas in salicin; 53 (59.6 per cent) were motile; only 10 (11.3 per cent) failed to form indol.

The distribution of the organisms, with regard to motility, dulcitol, glycerol, salicin and indol is given in table 4, and the coefficient of correlation for each pair of reactions is given in table 4A. For these strains motility is not distinctly correlated with

any other character. Dulcitol and glycerol are not correlated with each other, nor with indol and motility, but each has a high coefficient of association with salicin. The coefficient for dulcitol

TABLE 4

Showing correlation of characters among 89 sucrose negative strains of the coli group

		Motility		Indol		Dulcitol		Glycerol		Salicin	
		+	−	+	−	+	−	+	−	+	−
Motility	+	53		45	8	22	31	43	10	33	20
	−		36	34	2	11	25	26	10	18	18
Indol	+	45	34	79		29	50	61	18	51	28
	−	8	2		10	4	6	8	2		10
Dulcitol	+	22	11	29	4	33		27	6	28	5
	−	31	25	50	6		56	42	14	23	33
Glycerol	+	43	26	61	8	27	42	69		48	21
	−	10	10	18	2	6	14		20	3	17
Salicin	+	33	18	31		28	23	48	3	51	
	−	20	18	28	10	5	33	21	17		38

TABLE 4A

Coefficients of correlation for each pair of characters in table 4

	Motility	Indol	Dulcitol	Glycerol	Salicin
Motility		−.50	+.22	+.25	+.25
Indol	−.50		−.07	−.08	+1.00
Dulcitol	+.22	+.07		+.20	+.78
Glycerol	+.25	−.08	+.20		+.86
Salicin	+.25	+1.00	+.78	+.86	

with salicin is 0.78 and for glycerol with salicin is 0.86. Indol is also correlated with salicin; all of the 10 indol negative strains are also salicin negative. Differentiation is therefore made upon salicin which gives a sucrose negative, salicin positive subgroup

of 51 strains, and a sucrose negative, salicin negative subgroup of 38 organisms.

The sucrose-negative, salicin-positive subgroup (*B. coli*). The distribution of the 51 sucrose negative salicin positive strains on motility, dulcitol, and glycerol is indicated in table 5. 33 (64.9 per cent) are motile; 28 (54.9 per cent) form gas from dulcitol, and 48 (94.3 per cent) from glycerol. The extremely small proportion of glycerol negative strains (5.7 per cent) eliminates

TABLE 5

*Showing correlation of characters among 51 sucrose negative—**salicin positive strains** of the coli group.* (*B. coli*)

		Motility		Dulcitol		Glycerol	
		+	−	+	−	+	−
Motility	+	33		18	15	30	3
	−		18	10	8	18	
Dulcitol	+	18	10	28		25	3
	−	15	8		23	23	
Glycerol	+	30	18	25	23	48	
	−	3		3			3

* Coefficient of correlation for motility and dulcitol = 0.02.

this alcohol from further statistical consideration. From table 5 it is seen that motility and dulcitol are not correlated. Further subdivision on correlated characters is not feasible. This entire group then is regarded as the species *B. coli* and two varieties may be formed on motility—the motile *B. coli-communis* and the non-motile *B. coli-immobilis*.

The sucrose negative, salicin negative subgroup (*B. acidi-lactici*). Of the 38 organisms which were negative for both sucrose and salicin, 20 (52.7 per cent) were motile, 28 (73.7 per cent) formed indol, 21 (55.3 per cent) were positive with glycerol, while only 5 (13.2 per cent) formed gas from dulcitol as shown in table 6. From table 6A it appears that motility is correlated with dulcitol fermentation and indol, and has a slightly higher coefficient with

glycerol than has dulcitol. Indol has a slightly higher coefficient with dulcitol than motility, but the number of dulcitol positive strains is so small, that the coefficients observed cannot be relied upon. Indol and motility seem to be correlated (coefficient

TABLE 6

Showing correlation of characters among 38 sucrose negative, salicin negative strains of the coli group. (B. acidi-lactici)

		Indol		Motility		Dulcitol		Glycerol	
		+	−	+	−	+	−	+	−
Indol	+	28		12	16	2	26	14	14
	−		10	8	2	3	7	7	3
Motility	+	12	8	20		4	16	13	7
	−	16	2		18	1	17	8	10
Dulcitol	+	2	3	4	1	5		2	3
	−	26	7	16	17		33	19	14
Glycerol	+	14	7	13	8	2	19	21	
	−	14	3	7	10	3	14		17

TABLE 6A

Coefficients of correlation for each pair of characters in table 6

	Indol	Motility	Dulcitol	Glycerol
Indol		+.68	+.69	+.40
Motility	+.68		+.62	+.40
Dulcitol	+.69	+.62		+.34
Glycerol	+.40	+.40	+.34	

0.68), and their coefficients with glycerol are identical (0.40). In a preliminary report subdivision was made upon motility into the motile species *B. Gruenthal*, and non-motile *B. acidi-lactici*. It seems best, until more extensive collections are studied, that

all of the sucrose-negative, salicin-negative strains be included in the species *B. acidi-lactici* in which may be recognized two varieties, the motile *B. acidi-lactici* var. *Gruenthali* and the non-motile *B. acidi-lactici* var. *immobili.*

TABLE 7

Showing correlation of characters among 93 sucrose positive strains in the coli group

		Motility		Indol		Dulcitol		Glycerol		Salicin	
		+	−	+	−	+	−	+	−	+	−
Motility	+	77		69	8	58	19	49	27	56	21
	−		16	15	1	6	10	7	9	7	9
Indol	+	69	15	84		58	26	49	34	58	26
	−	8	1		9	6	3	7	2	5	4
Dulcitol	+	58	6	58	6	64		36	28	47	17
	−	19	10	26	3		29	20	8	16	13
Glycerol	+	49	7	49	7	36	20	56		41	15
	−	27	9	34	2	28	8		36	21	15
Salicin	+	56	7	58	5	47	16	41	21	63	
	−	21	9	26	4	17	13	15	15		30

TABLE 7A

Coefficients of correlation for each pair of characters in table 7

	Motility	Indol	Dulcitol	Glycerol	Salicin
Motility		−.27	+.67	+.40	+.54
Indol	−.27		+.05	−.42	+.28
Dulcitol	+.67	+.05		−.32	+.39
Glycerol	+.40	−.42	−.32		+.32
Salicin	+.54	+.28	+.39	+.32	

THE SUCROSE POSITIVE STRAINS OF THE COLI GROUP

Of the 93 organisms which fermented sucrose with gas formation, 77 (82.8 per cent) were motile; 84 (90.4 per cent) formed indol; and positive gas reactions were obtained as follows: dulcitol 64 (72.1 per cent); glycerol 56 (60.2 per cent) and salicin

63 (67.8 per cent). The distribution with respect to these characters and the correlation coefficients are shown in tables 7 and 7A respectively, where it is seen that motility correlates better with dulcitol than does any other of the characters. It also correlates best with salicin. Motility is the best correlated

TABLE 8

Showing correlation of characters among 77 sucrose positive motile strains of the coli group. (B. communior)

		Indol		Dulcitol		Glycerol		Salicin	
		+	−	+	−	+	−	+	−
Indol	+	69		53	16	43	25	51	18
	−		8	5	3	6	2	5	3
Dulcitol	+	53	5	58		32	26	41	17
	−	16	3		19	17	1	15	4
Glycerol	+	43	6	32	17	49		37	13
	−	25	2	26	1		27	19	8
Salicin	+	51	5	41	15	37	19	56	
	−	18	3	17	4	13	8		21

TABLE 8A

Coefficients of correlation for each pair of characters in table 8

	Indol	Dulcitol	Glycerol	Salicin
Indol		+.33	−.27	+.26
Dulcitol	+.33		−.87	−.22
Glycerol	−.27	−.87		+.09
Salicin	+.26	−.22	+.09	

character. There are thus two subgroups, a sucrose-positive, motile subgroup of 77 strains and a sucrose positive non-motile subgroup comprising 16 strains.

The sucrose positive motile subgroup (B. communior). Inspection of tables 8 and 8A shows that among the sucrose positive motile strains, neither indol production nor salicin fermentation is correlated with other characters. Gas formation from dulcitol

and glycerol shows a strong negative association. Those strains which failed to attack glycerol practically always fermented dulcitol. Thus 26 of 27 glycerol negative are dulcitol positive while 17 of 18 dulcitol non-fermenters, tested, formed gas from glycerol. To put it another way, inability to attack either of the alcohols is accompanied by fermentation of the other. Fermentation of either, however, yields but little information as to

TABLE 9

Showing correlation of characters among 16 sucrose positive, non-motile strains, of the coli group

		Dulcitol		Glycerol		Salicin	
		+	−	+	−	+	−
Dulcitol	+	6		4	2	6	
	−		10	3	7	1	9
Glycerol	+	4	3	7		5	2
	−	2	7		9	2	7
Salicin	+	6	1	5	2	7	
	−		9	2	7		9

TABLE 9A

Coefficients of correlation for each pair of characters in table 9

	Dulcitol	Glycerol	Salicin
Dulcitol		+.65	+1.00
Glycerol	+.65		+.80
Salicin	+1.00	+.80	

how the other will react. The desirability of subdividing on either glycerol or dulcitol to form two species is questioned. For the present the entire group of sucrose fermenting motile forms is designated as *B. communior* and two varieties may be formed on glycerol or dulcitol.

The sucrose-positive non-motile subgroup. Only 16 of the sucrose fermenters were non-motile, and only one of these failed to produce indol. Although the number of organisms is small, it is quite surprising that they should be so evenly divided with respect to gas formation from the test substances. Thus 6

(37.5 per cent) are positive with dulcitol, 7 (43.7 per cent) with glycerol, and 7 (43.7 per cent) with salicin. From tables 9 and 9A it is seen that salicin is the best correlated character. Of the seven salicin positive strains, 6 (85.8 per cent) attack dulcitol and 5 (71.5 per cent) glycerol. The characteristics of this group

TABLE 10

Distribution of organisms from different sources among the various species and varieties

		B. cloacae	B. aerogenes	B. communior	B. neapolitanus	B. coscoroba	B. coli communis	B. coli immobilis	B. acidi-lactici Grunthal	B. acidi-lactici immobili	Total
Soil	No	88	54	26	0	0	2	0	7	0	177
	%	49.7	30.5	14.7			1.1		4.0		
Horse	No	0	0	15	0	0	4	0	0	0	19
	%			79.0			21.0				
Sheep	No	0	0	16	0	5	1	0	0	0	22
	%			72.8		22.7	4.5				
Cow	No	0	0	6	4	0	9	0	1	0	20
	%			30.0	20.0		45.0		5.0		
Pig	No	0	0	9	0	1	11	1	9	0	31
	%			29.0		3.2	35.6	3.2	29.0		
Sewage	No	1	8	3	3	2	1	12	2	7	39
	%	2.6	20.5	7.7	7.7	5.1	2.6	30.8	5.1	17.9	
Man	No	0	0	2	0	1	5	5	1	11	25
	%			8.0		4.0	20.0	20.0	4.0	44.0	
Total		89	62	77	7	9	33	18	20	18	333

therefore resemble the *B. neapolitanus* of MacConkey's varieties. On the other hand, 7 (77.8 per cent) of the 9 salicin negative strains are negative for glycerol while all failed to ferment dulcitol. These are therefore the *B. coscoroba* of MacConkey's classification.

RELATION OF SPECIES TO SOURCE

Table 10 shows the distribution of the organisms from different sources among the various species and varieties. Species or varieties and habitat seem to be somewhat related.

B. aerogenes and *B. cloacae* were obtained only from soil and sewage and were not isolated from any of the animals tested. *B. cloacae* constituted 49.7 per cent of the soil and 2.6 per cent

of the sewage strains, while 30.5 per cent from soil and 20.5 per cent from sewage were *B. aerogenes.*

B. communior was isolated from all sources as follows: soil 14.7 per cent; horse 79 per cent; sheep 72.8 per cent; cow 30 per cent; pig 29 per cent; sewage 7.7 per cent and man 8 per cent. The relative abundance of *B. communior* among the lower animals and scarcity in man and sewage, may well be investigated further.

B. neapolitanus was present only in bovine feces and sewage, comprising 20 per cent of the bovine and 7.7 per cent of the sewage strains.

Of the 9 *B. coscoroba,* 5 were from sheep, 1 from pig, 2 from sewage, and 1 from man. 22.7 per cent of sheep, 3.2 per cent of pig, 5.1 per cent of sewage and 4 per cent of human strains fall in this species.

B. coli, like *B. communior* was isolated from all of the sources tested, but a rather distinct correlation with the source is observed with the varieties *B. coli-communis* and *B. coli-immobilis.* The former comprise 1.1 per cent of soil; 21 per cent of horse; 4.5 per cent of sheep; 45 per cent of cow; 35.6 per cent of pig; 2.6 per cent of sewage; and 20 per cent of human strains. *B. coli-immobilis* was not obtained from the soil, horse, sheep or cow, but it made up 3.2 per cent of the pig, 30.8 per cent of the sewage, and 20 per cent of the human strains.

B. acidi-lactici was not obtained from the horse nor sheep, and only rarely from the cow (5. per cent) or soil (4 per cent). The motile variety *B. acidi-lactici var. Gruenthali* was particularly abundant among the pig cultures (29 per cent) and rare in sewage (5.1 per cent) and man (4 per cent). The non-motile *B. acidi-lactici var. immobili* was restricted to man and sewage entirely, comprising 44 per cent of the human and 17.9 per cent of the sewage strains.

If subsequent and more extensive studies confirm these results the determination of species and varieties would have some bearing on the interpretation of the colon test.

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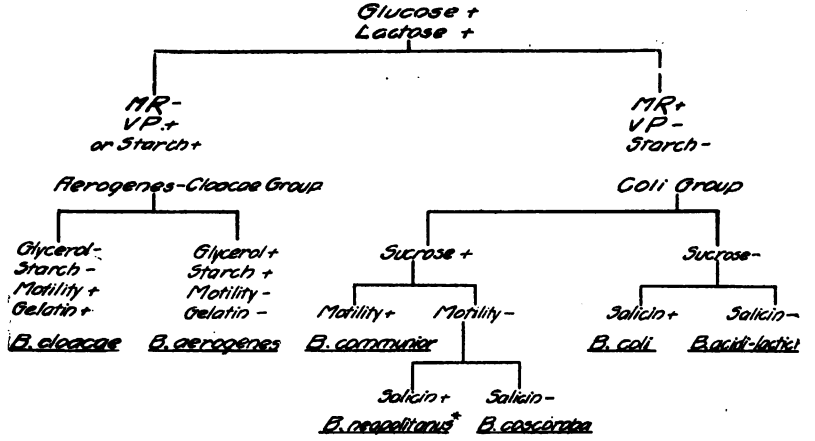
SUMMARY

From a statistical study of 333 coli-like bacteria isolated from soil, sewage, and the feces of various animals, the following classification is suggested:



* Designation as species questionable. Probably preferable to regard it as a variety of *B. communior*.

TABLE 11

Per cent of positive reactions

	V.P.	Indol	Gelatin	Motility	Starch	Inulin	Dextrin	Salicin	Raffinose	Sucrose	Dulcitol	Glycerol	No. of Strains
B. cloacae	100.0	16.8	91.0	100.0	4.5	4.5	30.4	98.0	93.3	96.7	15.7	9.0	89
B. aerogenes	100.0*	50.0	3.2	0.0	98.5	29.1	100.0	100.0	100.0	100.0	50.0	98.5	62
B. communior	0.0	89.6	0.0	100.0	0.0	0.0	0.0	72.8	94.8	100.0	75.4	63.7	77
B. neapolitanus	0.0	100.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	85.8	71.5	7
B. cascoroba	0.0	89.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	0.0	22.2	9
B. coli	0.0	100.0	0.0	64.7	0.0	0.0	0.0	100.0	11.8	0.0	55.2	94.3	51
B. acidi lactici	0.0	64.0	0.0	52.7	0.0	0.0	0.0	0.0	2.6	0.0	13.2	55.2	38

* Ten questionable reactions included.

The per cent of positive reactions of the different species is indicated in table 11. *B. neapolitanus* differs from *B. communior*

only with respect to motility, and it may therefore be well to regard it as a variety of *B. communior*. However *B. coscoroba* is so distinctly different from the other sucrose fermenters that its designation as a species seems justified. It should be borne in mind however, that the differentiation in this instance is based on only 9 individual cultures so that the correlations observed must not be over emphasized.

CONCLUSIONS

To treat all characters as of equal taxonomic significance leads to an infinite number of unstable varieties; a condition to be avoided.

Subdivision on correlated characters results in a small number of groups which possess considerable stability.

The species described are quite strikingly correlated with the source, and, if more extensive investigations confirm these observations, recognition of the various species may be of sanitary significance.

It is not supposed that the classification presented is the last word in the differentiation of coli-like bacteria, but it is hoped that if subdivision is to be made upon correlated characters—and there is much to commend such a procedure—the method described in this paper for the determination of the best correlated character, by a study of the coefficients of correlation, will be an aid to later investigators.

REFERENCES

BERGEY AND DEEHAN, S. J. 1908 J. Med. Research, **19**, 175.
DURHAM, H. E. 1901 J. Exper., **5**, 353.
FORD, W. W. 1903 Studies from the Rockefeller Inst. of Med. Res. 11.
HOWE, E. C. 1912 Science, N. S., **35**, 225.
JACKSON, D. D. 1911 Am. J. Pub. Health, **1**, 930.
JOHNSON AND LEVINE 1917 J. Bact.
KLIGLER, I. J., 1914 J. Infect. Dis., **15**, 135.
LEVINE, M. 1916 J. Infect. Dis., **19**, 773.
MACCONKEY, A. 1905 J. Hyg. **5**, 333; 1909 J. Hyg. **9**.
ROGERS, ET AL. J. Infect. Dis., 1914, **14**, 411; 1914, **15**, 100; 1915, **17**, 137.
SMITH, TH. 1893 The Wilder Quarter Century Book. 187.
YULE, 1916 An introduction to the theory of statistics.

Differentiation of B. Coli and B. Aerogenes on a Simplified Eosin-Methylene Blue Agar

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DIFFERENTIATION OF B. COLI AND B. AEROGENES ON A SIMPLIFIED EOSIN-METHYLENE BLUE AGAR

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For confirming the presumptive test for B. coli the mediums most frequently employed are litmus lactose agar and fuchsin sulphite (Endo) agar. It is becoming more apparent that the coli-like forms may be divided into two groups which are closely correlated with the source. One group (B. coli) is characteristic of fecal origin; the other (B. aerogenes and B. cloacae) is rare in feces, but constitutes the prevailing coli-like form in the soil and on grains. The standard litmus lactose and Endo agar may be employed to a slight extent for the differentiation of B. coli and B. aerogenes, but the differences between these types on these mediums (particularly L.L.A.) are not very clear-cut nor distinct. Better results are obtained with a modified Endo agar described elsewhere. A very excellent differentiation between the B. coli and B. aerogenes types has been obtained on a modification of eosin-methylene blue agar first described by Holt-Harris and Teague for the isolation of the typhoid group from feces. The medium is prepared in the following manner:

Distilled water	1000 c c
Peptone (Difco)	10 gm.
Dipotassium phosphate	2 gm.
Agar	15 gm.

Boil ingredients until dissolved and make up any loss due to evaporation. Place measured quantities in flasks and sterilize at 15 lbs. for 15 minutes.

Just prior to use add to each 100 c c of the melted agar, prepared as above, the following constituents:

Sterile (20%) lactose solution	1 gm. or 5 c c
Aqueous (2%) eosin (yellowish) solution	2 c c
Aqueous (1%) methylene blue solution	2 c c

Pour medium into petri dishes, allow them to harden in incubator and inoculate in the ordinary way. Smearing the surface with a glass rod seems preferable to the streaking method sometimes employed.

There is no adjustment of reaction and filtration of medium is not necessary.

B. typhosus and members of intermediate group also grow well on this medium producing transparent, colorless, or slightly amber colonies that are about one-half the size of B. coli.

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DIFFERENTIATION OF B. COLI AND B. AEROGENES ON EOSIN-METHYLENE BLUE AGAR

	B. coli	B. aerogenes.
Size:	Well isolated colonies are 3-4 mm. in diameter.	Well isolated colonies are larger than coli; usually 4-6 mm. in diameter or more.
Confluence:	Neighboring colonies show little tendency to run together.	Neighboring colonies run together quickly.
Elevation:	Colonies slightly raised; surface flat or slightly concave, rarely convex.	Colonies considerably raised and markedly convex; occasionally the center drops precipitately.
Appearance by Transmitted Light:	Dark almost black centers which extend more than ¾ across the diameter of colony; internal structure of central dark portion difficult to discern.	Centers deep brown; not as dark as B. coli, and smaller in proportion to the rest of the colony. Striated internal structure often observed in young colonies.
Appearance by Reflected Light:	Colonies dark, button-like, often concentrically ringed with a greenish metallic sheen.	Much lighter than B. coli.. Metallic sheen not observed except occasionally in depressed center when such is present.

RESULTS WITH PURE CULTURES

A number of pure cultures were employed to test the value of this medium for the differentiation of B. coli, B. aerogenes, and members of the typhoid and paratyphoid groups.

Of 22 cultures of B. aerogenes all but 3 gave the characteristic reactions. Of these 3 cultures, 1 resembled B. coli on the eosin-methylene blue agar, another failed to produce a black center, and the 3rd showed a slight metallic lustre, but did not resemble B. coli closely.

Of 35 cultures of B. coli tested, 29 were typical. Six did not show a distinct metallic lustre, but were typical in other respects.

There were 23 strains of B. cholerasuis, B. paratyphosus, and B. typhosus tested. One strain of B. paratyphosus A did not grow. All other strains of the intermediate group developed typical transparent colonies.

RESULTS OBTAINED WITH WATER SAMPLES

The differentiation of pure strains seemed to be so marked and distinct that it was thought the medium might be employed for confirmation of the presumptive test for B. coli, and that it might be pos-

sible to differentiate B. coli from B. aerogenes simultaneously with confirming the presumptive test. For this purpose the following experiment was carried out.

Seven samples of water from different parts of the Iowa river, one of sewage, one of a small creek, and one from a stagnant body of water were plated out directly on litmus lactose agar and inoculated into lactose broth. After 24 hours' incubation 10 acid colonies were fished from the litmus lactose agar plates of each sample for further study. The lactose broth tubes were plated out after 48 hours' incubation onto eosin-methylene blue agar and onto litmus lactose agar. After 24 hours' incubation 10 colonies were fished from these litmus lactose agar plates of each sample for further observation. From the eosin-methylene blue plates made from the preliminary lactose broth tubes colonies which resembled B. coli or B. aerogenes were fished and tentatively designated as such, with a view to determining the accuracy and reliability of the plate differentiation.

All colonies fished from litmus lactose agar were reinoculated into lactose broth and after 24 hours' incubation were plated out on eosin-methylene blue agar. From each plate was picked a well isolated colony which was designated as B. coli or B. aerogenes. These designations were then checked by growing the organisms in Clark and Lubs medium and testing with the methyl-red and Voges-Proskauer reactions.

CULTURES OBTAINED DIRECTLY FROM LITMUS LACTOSE AGAR

Of the 10 water samples examined 1 did not show acid colonies by direct plating on litmus lactose agar. Of the 90 acid colonies fished, 3 were found to be lactose nonfermenters. Thirty-three cultures were regarded, from their appearance on eosin-methylene blue agar, as B. aerogenes. Of these, 24 (72%) gave the Voges-Proskauer reaction. Seven cultures were diagnosed tentatively as questionable but probably B. aerogenes but none of these gave a Voges-Proskauer reaction.

Eight cultures were regarded as questionable, but probably B. coli, of which 6 (75%) did not give the Voges-Proskauer reaction. Thirty-nine cultures were designated from their appearance on eosin-methylene blue agar, as B. coli, and all were confirmed, as none gave the Voges-Proskauer reaction.

Of 40 cultures which were regarded tentatively as B. aerogenes or probably B. aerogenes 24 (60%) were correct. Of 47 cultures regarded as B. coli or probably B. coli, 45 (95.8%) were correct.

CULTURES OBTAINED FROM LITMUS LACTOSE AGAR AFTER PRELIMINARY ENRICHMENT IN LACTOSE BROTH

After elimination of a few strains which proved to be other than coli forms, 85 cultures which were isolated from litmus lactose agar plates made from the lactose broth preliminary enrichment tubes were

smeared onto eosin-methylene blue agar for differentiation. One organism was regarded as probably B. aerogenes and on confirmation proved to be B. coli. Of 35 organisms recorded as B. aerogenes 34 (97%) gave the Voges-Proskauer reaction. Forty-nine organisms were tentatively designated as B. coli or probably B. coli and all proved to be negative for the Voges-Proskauer reaction.

With organisms isolated from this group then, 34 out of 36 cultures regarded as B. aerogenes were confirmed as such while every one of 49 strains regarded as B. coli was correct.

CULTURES OBTAINED FROM EOSIN-METHYLENE BLUE AGAR AFTER PRELIMINARY ENRICHMENT IN LACTOSE BROTH

Fifty-two cultures were fished, 26 of which were regarded as B. aerogenes and the remaining as B. coli. Of the 26 supposedly B. aerogenes strains all gave the Voges-Proskauer reaction. Two of the strains regarded as B. coli also gave the Voges-Proskauer reaction. Thus 100% of the B. aerogenes strains and 92.4% of the B. coli strains were correctly differentiated on eosin-methylene blue agar.

Results on all cultures isolated may be summarized as follows:

Tentatively regarded as B. coli from appearance of eosin-methylene blue agar	122
Correctly designated as indicated by negative Voges-Proskauer reaction	118
Per cent. confirmed	96.9
Tentatively regarded as B. aerogenes from appearance on eosin-methylene blue agar	102
Correctly designated as indicated by positive Voges-Proskauer reaction	84
Per cent. confirmed	82.4

CORRELATION OF VOGES-PROSKAUER AND METHYL-RED REACTION

In previous work a marked correlation was observed between the Voges-Proskauer and methyl-red reactions. Strains of coli-like forms which were acid to methyl-red characteristically did not give a test for acetyl-methyl-carbinal (V-P negative); while those reacting alkaline to methyl-red gave a positive Voges-Proskauer test. These observations were confirmed by Greenfield,[1] Hunter,[2] Clark,[3] Hutton,[4] Rettger and Burton[5] and others.

In this group of strains studied a similar correlation was observed. The relation between the Voges-Proskauer and methyl-red reactions is indicated in the following table:

	Methyl-red		
V. P.	+	N.*	—
+	2	2	84
—	121	13	2

* In previous work neutral reactions to methyl-red have been grouped with the acid strains.

It is seen from the table that there is an excellent correlation between the two reactions. Placing the neutral reacting strains with the acid group as was done in previous studies, we find that 84 (97.8%) of 86 methyl-red negative strains give the V. P. reaction; while of 136 strains not giving the V. P. reaction 134 (98.7%) were acid or neutral to methyl-red.

In this series of cultures the V. P. reactions were more clear-cut than the methyl-red test but we have worked with collections in which the reverse was true. It seems best to employ both the V. P. and methyl-red tests and to repeat if the results do not agree.

It is interesting to note that of 87 cultures isolated from litmus lactose agar plates made directly, 29.9% proved to be B. aerogenes; whereas of 85 cultures isolated from litmus lactose agar plates made after preliminary enrichment for 48 hours in lactose broth, 40% proved to be B. aerogenes. This indicates the correctness of the contention of Race and others that preliminary enrichment tends to an overgrowth of B. aerogenes types.

Organisms other than B. coli and B. aerogenes grow quite well on this medium and several have been observed to produce small blue centers; but the appearance is so distinct from B. coli and B. aerogenes that once having observed the true types there should be no mistake. Just what these other forms are has not been determined but several have been isolated and will be reported on in a future report. They produce very small colonies with pinpoint light blue or delf-blue centers. The color is very different from the brownish black appearance of the B. coli and B. aerogenes types. Perhaps the introduction of some inhibitory dye into the medium will make it even more reliable for the isolation and differentiation of B. coli and B. aerogenes and confirmation of the presumptive test.

[1] Jour. Infect. Dis., 1916, 19, p. 647.
[2] Jour. Bacteriol., 1917, 2, p. 585.
[3] Jour. Biol. Chem., 1917, 30, p. 209.
[4] Jour. Infect. Dis., 1916, 19, p. 606.
[5] Ibid., 21, p. 162.

Dysentery and Allied Bacilli

MAX LEVINE

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DYSENTERY AND ALLIED BACILLI

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From the Laboratory of the Central Medical Department, A. E. F., Dijon, France, and the Army Medical School, Washington, D. C.

In France we not infrequently experienced difficulties in growing dysentery bacilli and work was therefore begun (1) to differentiate the true dysentery bacilli, which are universally recognized as pathogenic, from the atypical or dysentery-like organisms (B. ambiguus, B. alkalescens, and B. dispar) many strains of which are nonpathogenic and whose etiologic significance is questionable; (2) to devise a more dependable, and if possible more simple medium, than the nutrient agar (phenolphthalein titration) for the isolation of dysentery bacilli.

The nomenclature in the group of dysentery bacilli has become quite confused. In this paper the following will be adhered to: B. dys. Shiga corresponds to the original Shiga-Kruse mannite negative type. The term B. flexneri includes both the B. dys. Flexner and Y types, and when possible it will be qualified with the race of the strain, such as V, W, X, Y or Z. The terms B. dys. Flexner and B. dys. Y are used in their old significance.

Serologic tests and studies on classification were beyond the scope of the investigation. Agglutination with stock Flexner and Y serums were carried out with 59 cultures. Acid production in a number of sugars and other fermentable substances, as well as the reactions in milk and the indol test, were observed on all the stains.

A total of 111 cultures were considered in this study. These were distributed as follows: B. dys. Shiga, 17; B. ambiguus, 5; B. flexneri, 60; B. alkalescens, 12; B. dispar, 11; miscellaneous, 6.

The Shiga cultures, with one exception, were stock strains found at the Central Medical Laboratory or the Army Medical School; several were duplicates.

The ambiguus strains included 3 (67, 68 and 69) from Dr. Andrews, St. Bartholomew's Hospital, London. One (4) was found at the Central Medical Laboratory marked B. dys. Shiga Fletcher vaccine stain, and another (101) obtained from the Army Medical School and probably a duplicate of (4), was marked B. dys. Shiga Fletcher 1. Serologic tests were not made with (101). The other (4) failed to agglutinate with several Shiga serums, and as both were positive for indol they are here considered as B. ambiguus.

The 60 cultures of B. flexneri include strains isolated during the war and also standard stock cultures. Included in this group are the old Flexner and Y types and authentic strains of the English groups V, W, X, Y, Z, VZ and WX, which were sent me by Dr. Andrews.

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There were 12 strains of B. alkalescens and 11 of B. dispar. These were received from Dr. Andrews or freshly isolated in laboratories of the A. E. F.

Of the 6 miscellaneous strains two (37 and 57) were marked B. ambiguus. They produced a green fluorescence in broth and on gelatin and were so different culturally from the strains of B. ambiguus received from Dr. Andrews that it seems they should not be considered as of the same group. Two cultures (48 and 108) were marked B. dys. Sonne. They did not agglutinate with the Flexner or Y serums available. Lactose was fermented with acid formation and then became alkaline. Milk was turned acid but not coagulated. These cultures resemble markedly some of the B. dispar of Andrews, at least culturally. One strain, 3, supposedly a Shiga, produced acid from sucrose and gave indol. It was not agglutinated with a Shiga serum. Another strain, 97, differed from all of the other cultures studied in that it fermented the glucoside salicin with a strong acidity in 24 hours.

AGGLUTINATION WITH FLEXNER AND Y SERUMS

Agglutination was made with living 24-hour broth cultures of 59 strains. The strains of B. dys. Shiga, B. alkalescens, and B. dispar were not agglutinated by either of the serums. B. dys. Sonne (48) and one of the English B. flexneri Z race (53), were also not agglutinated. It was noticed that the Z and X races of B. flexneri were only agglutinated in the low dilutions, and that (13 and 38) the original Mt. Desert Y and the Oxford Y strain, respectively, were not agglutinated even in 1:100 by the Y serum employed. From these observations it appears quite evident that what is regarded as the Y type of dysentery in different laboratories is not of the same serologic group.

BIOCHEMICAL REACTIONS (TABLE 1)

All strains were gram-negative short rods, and nonmotile as determined in semisolid agar (0.5% agar in broth).

TABLE 1

ACID PRODUCTION AND INDOL (PERCENTAGE OF POSITIVE REACTIONS) BY DYSENTERY AND CLOSELY ALLIED BACILLI

Organism	No. of Strains	Mannitol	Lactose	Glycerol*	Dextrin	Dulcitol	Sucrose	Xylose	Raffinose	Rhamnose	Indol
B. dys. Shiga.....	17	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B. ambiguus.....	5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0
B. dys. flexneri†.....	59	100.0	0.0	0.0*	40.0	0.0	64.4	0.0	79.7	16.9	83.1
B. alkalescens.....	12	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	100.0
B. dispar.....	11	100.0	100.0	81.3	0.0	18.2	81.8	81.8	91.9	100.0	81.8

* Slight acidity in 5-7 days but more alkaline than P_H 7.0.
† Includes all mannite fermenting true dysentery bacilli.

Tests for acid production were made on glucose, mannitol, lactose, glycerol, sucrose, dextrin, arabinose, dulcitol, rhamnose, xylose, raffinose and salicin. The medium employed consisted of 1% peptone, and 0.4% dipotassium phosphate with 1% of the test material. The rosolic acid-china blue mixture of Bronfenbrenner was the indicator. Incubation was at the body temperature, and observations were made daily for 7 days.

The indol reaction was determined from peptone water after 5 days' incubation by the nitroso-indol reaction. Litmus milk was observed for 13 days

Table 1 indicates that the 5 main types of dysentery and dysentery-like organism may be readily differentiated by fermentation and indol reactions

Thus B. dys. Shiga and B. ambiguus may be distinguished from the others (B. flexneri, B. alkalescens, and B. dispar) by the inability of the former to give acid from the alcohol mannitol. They differ from each other in that B. ambiguus forms indol and ferments rhamnose.

B. flexneri may be differentiated in a large proportion of instances from B. alkalescens and B. dispar by the reaction in glycerol and xylose. None of the Flexner strains produced acid from xylose, whereas this substance was fermented vigorously by 21 of 23 strains of B. alkalescens and B. dispar. Differentiation by glycerol fermentation was not so distinct, as a number of the Flexner strains produced a small amount of acid. Quantitative studies showed that this acidity was never beyond the true neutral point P_H 7.0. in 5 days. With the indicator employed, however, the results might be confusing in inexperienced hands.

B. flexneri differs also from B. alkalescens and B. dispar in the milk reaction. The former produces a faint acidity in litmus milk, which reverts very slowly, if at all, to a neutral reaction in from 10-13 days. B. alkalescens, on the other hand, reverts relatively rapidly, from 4-8 days, to a distinct alkaline reaction, while B. dispar becomes progressively more acid, eventually coagulating the medium. Unfortunately the milk reaction has not given concordant results in the hands of different observers, many recording distinct alkalinity and others coagulating with true dysentery strains of B. flexneri type.

It remains to differentiate B. alkalescens from B. dispar. The milk reaction has been referred to. The objectionable features of this reaction are the variability of different batches of milk and slowness of the test. The lactose fermentation of B. dispar, although distinct, is often long-delayed. Table 1 shows that although there is some overlapping, the two organisms are markedly different when groups of characters rather than single reactions are considerel. Thus B. alkalescens does not form acid from lactose, sucrose or raffinose, but attacks dulcitol vigorously, while B. dispar rarely ferments dulcitol, but does form acid from lactose and most always from sucrose (81.8%) and raffinose (91.9%). B. alkalescens seems to be a very homogenous group. B. dispar probably consists of several varieties. The indol-negative, xylose-negative variety of B. dispar corresponds culturally to the strain isolated by Sonne in Denmark.

VARIETIES OF B. FLEXNERI

A number of subdivisions of the mannite fermenting dysentery strains on serologic and biochemical reactions have been proposed in the past. The probable untenability of B. dys. Y as distinguished from B. dys. Flexner has already been referred to. The differentiation of B. flexneri by the English War Committee as determined by careful absorption tests into V, W, X, Y and Z races appears much more acceptable and desirable.

The value of differentiation of this group on fermentation reactions has fallen into disrepute of late. Thus the fermentation of maltose, sucrose and dextrin, which were formerly emphasized as differentiating varieties of mannite fermenting dysentery strains, is about to be discarded. Maltose was not employed in this study as it was considered unreliable on account of the difficulty in obtaining a product entirely free from glucose, and the ease with which it decomposes on sterilization. Of the tests tried with 59 strains of B. Flexneri the following positive results were obtained with substances that might be of value for subdivision: sucrose, 64.4%; dextrin, 40%; rhamnose, 16.9%; raffinose, 79.7%; and indol, 83.1%. The correlation coefficients for each

pair of characters is given in table 2[1] which shows rhamnose correlates best with the other characters. Subdividing on rhamnose, two groups are obtained as follows:

	Strains	Percent Positive			
		Sucrose	Dextrin	Raffinose	Indol
Rhamnose +	10	80	70	0	100
Rhamnose —	49	60	32	94	78

TABLE 2

CORRELATION COEFFICIENTS FOR FERMENTATIVE CHARACTERS

	Sucrose	Dextrin	Rhamnose	Raffinose	Indol
Sucrose	.....	+0.62	+0.43	—0.06	+0.35
Dextrin	+0.62	.....	+.065	—0.46	+0.50
Rhamnose	+0.43	+0.65	.....	—1.00	+1.00
Raffinose	—0.06	—0.46	—1.00	.....	—0.45
Indol	+0.35	+0.50	+1.00	—0.45	

Raffinose fermentation is particularly interesting. Of 30 strains in the rhamnose-negative subgroup which fermented sucrose, all attacked raffinose; but, of 8 sucrose fermenters in the rhamnose-positive subgroup none attacked the trisaccharid. The source of the 10 rhamnose fermenting strains was: Strain 26 was isolated at the Cent. Med. Dept. Lab. from a patient and diagnosed as probably B. dys. Y; (60 and 61) were isolated at Lab. 1, A. E. F., from a patient and carrier, respectively, and reported as B. dys. Flexner and B. dys. Y. and sent in for further identification. As the foregoing diagnoses were based merely on the two serums available—Flexner and Y—the designations should not be accepted as final. It would be desirable to know to which race of Flexner bacilli they belong. The remaining 7 strains were received from Dr. Andrews and Dr. Inman of London. One, 94, was labelled B. flexneri Y race which is a sort of composite of the V, W, X and Z races. The other 6 strains were all B. flexneri Z race. Thus there seems to be a correlation between rhamnose fermentation and the Z race of B. flexneri. If subdivision is to be made at all on fermentation reactions, then it appears that rhamnose would be the logical choice.

ACID PRODUCTION FROM GLUCOSE

In order to devise a medium for the differentiation of B. alkalescens and B. dispar from the other dysentery or dysentery-like strains, the effects of various constituents of a selected medium on the rate of acid production and reversion were studied. Ten cultures, 2 B. dys. Shiga, 2 B. dys. Y, 2 B. dys. Flexner, 2 B. alkalescens, and 2 B. dispar were chosen for study.

Concentration of glucose.—The medium consisted merely of peptone (Difco) 1% dipotassium phosphate 0.4%, and glucose in varying amounts 0.0 to 0.5%, prepared in the following manner: To 1,000 c c of distilled water in a flask was added 10 gm. of peptone, 4 gm. of dipotassium phosphate, and the flask was then heated until the contents were dissolved (about 20-30 minutes). The medium was then filtered through paper and enough of a freshly prepared 10% glucose solution was added to give the desired concentration of the carbohydrate. The medium was tubed (about 20-25 c c) and sterilized in the autoclave 10 minutes at 10 pounds, after which it was incubated to eliminate unsterile tubes.

[1] See Levine, Jour. Infect. Dis., 1918, 3, p. 253.

Inoculation was made with 0.1 c c of a 24-hour broth culture with incubation at body temperature.

H-ion concentration was determined daily for 4 days by withdrawing 1 c c of the culture into 4 c c of neutral distilled water (P_H 7.0) in a clean, flat bottomed test tube, and after adding the required amount of an appropriate indicator, the color was matched with H-ion standards. Great difficulty was encountered in obtaining neutral distilled water in the laboratory in France. It was found, however, that the error introduced by the neutralization of ordinary distilled water with a small amount of sodium hydroxid was only about 0.1, which was within the limits of error in reading. Such neutralized water had to be freshly prepared and quickly utilized.

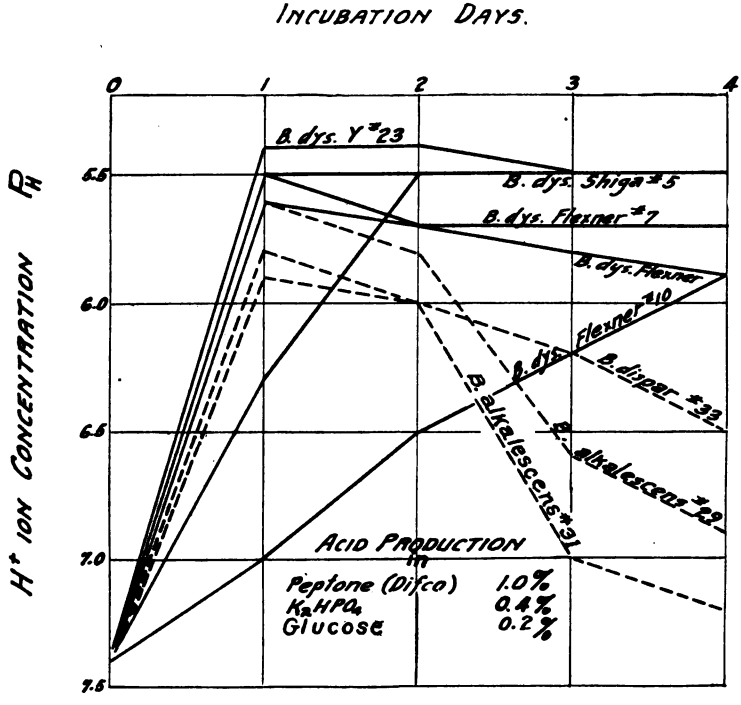

Chart 1.—Effects of peptone, dipotassium phosphate and glucose on the rate of acid production and reversion.

It was concluded (1) that with 1.0% peptone and 0.4% dipotassium phosphate, the employment of 0.3% or more glucose was undesirable for the purpose of differentiating B. alkalescens and B. dispar from the true dysentery bacilli; (2) that in the absence of glucose B. alkalescens and B. dispar form alkali more rapidly than the true dysentery strains (Shiga and B. flexneri); (3) that with 0.1% glucose there is reversion among the true dysentery strains, but B. alkalescens and B. dispar revert much more rapidly; (4) that with 0.2% glucose reversion among the true dysentery cultures was greatly inhibited, whereas B. alkalescens and B. dispar showed a marked alkali production after the primary acidity, as is shown in chart 1.

Concentration of Peptone.—The following experiment was made in Washington to determine the effect of the concentration of peptone on acid production and reversion:

Three batches of medium (0.2% glucose, 0.4% dipotassium phosphate, and 1.0%, 1.5% and 2.0% peptone respectively) were prepared as described; 7 c c portions were placed in tubes, autoclaved at 10 pounds for 10 minutes and incubated for 48 hours to eliminate unsterile tubes.

Seven tubes of each medium were inoculated from 24-hour cultures of organisms and incubated at 37 C.

Acidity determinations were made by the comparator in place of the dilution method previously described. Two duplicate cultures were taken and to one was added 0.3 c c of an appropriate indicator and the color matched with standards, the duplicate tube being employed to correct the error due to the color and turbidity of the culture medium in the comparator test. This tube was reincubatel and employed for this purpose in acidity determinations on subsequent days.

The concentration of peptone did not influence acidity production nor reversion of the true dysentery bacilli nor of B. ambiguus, but that with B. alkalescens and B. dispar reversion was much more rapid with 1.5% peptone than when 1.0% peptone was employed. Increasing the concentration to 2.0% did not further increase the rate of reversion.

Comparing the results with 1.0% peptone with those previously obtained in the original experiment in France, reversion was somewhat delayed in the new series. Although an adequate explanation is not available, it is felt that the difference is probably due to a difference in the actual concentration of glucose. The glucose available overseas was probably not thoroughly anhydrous.

Aeration seems to increase the rate of alkali production, after the primary acidity, in the case of B. alkalescens and B. dispar.

To determine whether the differentiation indicated in the quantitative observations could be applied qualitatively, each organism was inoculated in duplicate into the peptone phosphate medium containing as indicators 1% of a 0.5% phenol-red and 1% of a 0.2% brom-cresol-purple, respectively, and incubated at 37 C. Records of acidity were made daily.

With exception of (37 and 57), which have been referred to as probably misplaced in this group, and which remained alkaline throughout the experiment, all other cultures were distinctly acid to both indicators after 24 hours' incubation. With brom-cresol-purple as the indicator, all cultures of B. alkalescens and B. dispar showed reversion to distinct purple-blue color, as did also one of the B. dys. Sonne after 3 days' incubation. The true dysentery strains and B. ambiguus were yellow or brownish in color. On further incubation (6 days), the other strain of B. dys. Sonne and one B. flexneri became distinctly alkaline and a number of the true dysentery cultures began to show some reversion, thus obscuring, though not eliminating, the differentiation.

With phenol-red, on the other hand, all cultures of B. dys. Shiga and B. ambiguus, and all but one of B. flexneri were distinctly acid for 6 days. The 12 B. alkalescens strains were distinctly alkaline. Two of the 11 B. dispar were neutral, the others distinctly alkaline. One B. dys. Sonne was neutral and another alkaline.

Rate of Acid Production.—It was observed that glucose was attacked more rapidly by B. alkalescens and B. dispar than by the other organisms of this collection. Inoculation was from 24-hour broth cultures (0.1 c c to 30 c c of

medium) and H-ion determinations were made by the dilution method. In chart 2 the data are shown graphically.

The rate of acid production was observed qualitatively by the use of brom-cresol-purple and in some instances with the china-blue rosolic acid indicator. Inoculation was made from 24-hour agar slants; incubation was at 37 C. in the ordinary manner; acidity was recorded after 6 hours. At this time all strains of B. alkalescens and B. dispar, one B. flexneri and the 2 B. dys. Sonne were distinctly acid as indicated by a distinct or dirty yellow with brom-cresol-purple. All other strains produced acid less rapidly. They showed a distinct purple (more alkaline than P_H 6.3) with brom-cresol-purple and with the china-blue mixture were colorless or light blue.

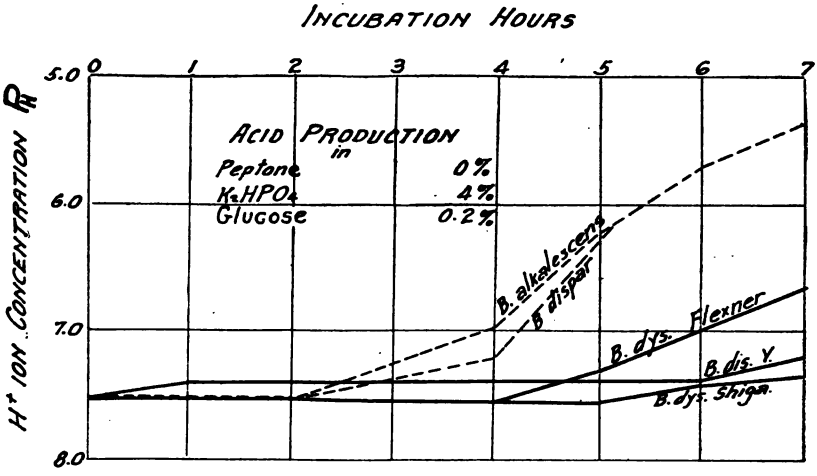

Chart 2.—Rate of acid production: Inoculations made from 24-hour broth cultures and H-ion determinations made by dilution method.

It may be concluded from the observations of glucose fermentation that B. alkalescens and B. dispar produce acid more rapidly and then revert to a distinctly alkaline reaction that may be indicated qualitatively by phenol-red or brom-cresol-purple. The use of brom-cresol-purple, however, would require experience and care, whereas phenol-red necessitates a prolonged period of incubation. The most desirable indicator for qualitative differentiation would be one which changes at P_H 6.5 showing a distinct coloration on the alkaline side.

A SIMPLIFIED SOLID MEDIUM FOR GROWTH AND ISOLATION OF DYSENTERY BACILLI

After a number of preliminary experiments it was found that by the addition of a small amount of glucose (0.03-0.05) to peptone-phosphate agar, growths as luxuriant, if not more so, than on nutrient agar could be obtained. As facilities for determination of the optimum H-ion concentration were not available at the time in France a series of experiments were carried out to determine the optimum concentration of dipotassium phosphate for growth of dysentery bacilli in a medium not requiring any further adjustment of reaction.

The medium consisted of 1.0% peptone, 0.1% glucose and 0.2 to 0.7% phosphate. Inoculation of the agar plates was made from a 24-hour broth culture. A concentration of 0.4-0.5% of the phosphate gave best results with 6 cultures examined. It is interesting in this connection to note that the titratable acidity with phenolphthalein was in each instance + 0.7%. The H-ion concentration was much more varied, probably 7.1 with the 0.2% of the phosphate and 7.8 with the 0.7% of the buffer salt, as indicated by subsequent experiments.

The influence of the H-ion concentration on the growth of the dysentery bacilli seems marked on solid medium. It has been my experience that in liquid medium the effect of the H-ion concentration is not so evident.

Experiments on the effect of the concentration of dipotassium phosphate repeated with 39 strains using 3 concentrations of the salt (0.2, 0.45 and 0.7%, respectively), showed that:

1. The phenolphthalein titration is a poor index of the true acidity of the medium. The variation in the titrable acidity was close to the limit of experimental error, whereas the difference in H-ion concentration with the different quantities of phosphate was marked and distinct.

2. The optimum reaction is not the same for all strains of dysentery. Two (5.1%) grew best with the largest quantity of phosphate, four (10.2%) with the least amount of phosphate and twelve (30.7%) show their optimum growth when 0.45% of dipotassium phosphate was used. Seventeen (43.6%) did equally well on all of the 3 mediums. Considering all cultures, we find 33 (84.7%) to have done as well or better with 0.45% phosphate than on either of the other concentrations of this salt. The H-ion concentration with 0.4% of the phosphate is generally 7.4 or 7.5. This quantity was selected as probably the most reliable and desirable.

Choice of Indicator.—A distinct and noninhibitory indicator is an important adjunct to the successful isolation of dysentery bacilli. It was hoped that the eosin and methylene-blue combination of Holt, Harris and Teague, which was found so valuable in water work, might be successfully employed, particularly as it was reputed to be noninhibitory. Thirty-nine strains of dysentery bacilli were inoculated on agar with and without the indicator from a 24-hour peptone phosphate culture.

The composition of the medium was:

Agar	1.5%
Peptone	1.0%
Dipotassium phosphate	0.4%
Glucose	0.1%
Indicator per 100 c c of above	
Eosin 2% yellowish aq	2.0 c c
Methylene-blue 0.5% aq	2.0 c c

(The P_H of this medium was 7.5).

B. dys. Shiga was markedly inhibited. A slight growth was observed on prolonged incubation (48-72 hours). Sixteen, or 50%, of the mannite fermenting dysentery strain were partially inhibited.

Of a number of indicators tried, the china-blue rosolic acid mixture was found to be the least inhibitory when working with pure cultures. Similar results were obtained with artificial suspensions of dysentery organisms in normal stools.

SUMMARY AND CONCLUSIONS

Observations made on 111 strains of dysentery and dysentery-like organisms indicate:

1. The strains of B. dysenteriae Y used in different laboratories are not of the same serologic group.

2. The main groups of the dysentery and closely allied bacilli, B. dys. Shiga, B. flexneri, B. ambiguus, B. alkalescens and B. dispar, are readily differentiated by fermentation reactions. B. dys. Sonne appears to be intermediate between B. dispar and B. flexneri.

3. Subdivision of B. flexneri on fermentation reactions is not advisable, but the flexneri Z race seems to be characterized by acid production from rhamnose. This character is also strikingly correlated with an inability to attack raffinose when sucrose is fermented.

4. B. alkalescens and B. dispar form acid from glucose rapidly in a medium containing 1.5% peptone, 0.4% dipotassium phosphate, and 0.2% glucose, then revert rapidly to an alkaline reaction. B. dys. Shiga, B. flexneri and B. ambiguus form acid less rapidly and remain permanently acid or revert slowly.

5. Dyes, such as eosin and methylene-blue, the fuchsin-sulphite indicator, and excess of rosolic acid or china-blue were found to inhibit many strains of dysentery, particularly the Shiga type.

6. The following medium is suggested for isolation work:

Distilled water	1,000 c c
Agar	15 gm.
Peptone	10 gm.
Dipotassium phosphate	4 gm.

To each 100 c c of the melted medium add before using:

Lactose, 20% solution	5.0 c c
Glucose, 5% solution	1.0 c c
Rosolic acid (1.0% in 90% alcohol)	1.0 c c
China-blue (0.5% in water)	1.0 c c

The H-ion concentration of this medium, which requires no adjustment of reaction and does not need to be filtered when used on plates, is 7.4 to 7.5.

With the compliments of the author

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A FACULTATIVE SPORE-FORMING LACTOSE-FERMENTING ORGANISM FROM IOWA SURFACE WATERS, (B. MACERANS)[1]

BY JACK J. HINMAN, JR.,[2] AND MAX LEVINE[3]

The occasional presence of sporing lactose-fermenters in water capable of growing aerobically has been reported by Meyer, Ewing, Ellms, Perry and Monfort, and Hall and Ellefson, but very little is known as to the source or biology of these forms.

In the course of routine water analyses at the Iowa State Water Laboratory it appeared that, with chlorinated surface waters the proportion of unconfirmed presumptive tests was excessive, when using preliminary enrichment in lactose broth, and eosin methylene blue agar for confirmation. With litmus lactose agar, however, atypical colonies were not infrequently obtained, which often formed gas when fished to lactose broth and which would therefore be regarded as members of the colon group, on the basis of the Treasury Department Standard. These organisms were invariably negative for gas formation in lactose bile. It occurred to us that possibly aerobic sporing lactose bacilli might be responsible, for a part at least, of these atypical reactions, and attempts were made to isolate them.

Table 1 has been prepared to show the frequency with which presumptive tests upon filtered, chlorinated Iowa city waters were found to be organisms which could not be confirmed as B. coli or B. aerogenes. It appears that those waters which were taken from the Iowa River, or from the Mississippi River below the junction of the Iowa with the Mississippi, are particularly likely to contain these non-confirmed organisms. This apparent peculiarity may be due, however, to the greater number of samples examined from the cities of Burlington, Iowa City and Keokuk where such water is handled.

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From two sources, Iowa City and Burlington, Iowa, 14 pure cultures have been isolated and studied as to their morphological, cultural and other characteristics. Considerable difficulty was encountered in effecting separation from non-fermenting, sporing

TABLE 1

Fermentation tests on treated Iowa city waters, March 3, 1914, to December 30, 1920

CITY	1 CC. WATER				10 CC. WATER			
	B. coli	B. aerogenes	Positive presumptive tests not confirming	No gas formation	B. coli	B. aerogenes	Positive presumptive tests not confirming	No gas formation
Burlington	22	2	41	651	71	22	584	1852
Cedar Rapids	0	1	7	11	3	4	21	23
Centerville	9	1	14	54	33	5	40	37
Chariton	2	0	2	11	12	1	12	12
Council Bluffs	0	0	2	4	0	0	8	8
Creston	3	2	5	27	11	11	12	34
Davenport	2	0	2	25	12	0	28	80
Fairfield	1	0	5	8	1	0	2	1
Ft. Madison	3	1	2	11	14	7	10	17
Iowa City	35	2	312	3868	62	6	492	1147
Keokuk	11	0	22	101	56	8	258	135
Lenox	3	1	2	2	4	8	2	0
Oskaloosa	0	0	1	7	0	0	5	7
Ottumwa	0	0	4	8	0	0	5	10
Storm Lake	3	0	0	16	18	3	2	21
	94	10	421	4804	297	75	1481	3384

Total number of fermentation tubes.....10,566
Total number positive..... 2,378
Total number positive, non-confirming..... 1,902
Per cent positive tubes non-confirming..... 80 per cent
Per cent all tubes non-confirming positive..... 18 per cent
Burlington, Iowa City, Keokuk:
Per cent positive tubes non-confirming..... 85.3 per cent
Per cent all tubes non-confirming positive..... 17 per cent

aerobes, which seemed to grow associatively. The method which proved most successful was (1) to grow in lactose (Andrade) broth, (2) to plate on lactose (Andrade) agar as soon as acid developed, (3) fish acid colonies to lactose (Andrade) agar slants (inoculate sur-

face and butt) and incubate the latter for 72 hours observing daily. If pure, a transparent growth with acid on slant and acid and gas in butt will be observed. If non-fermenting spore-formers are present the surface growth becomes opaque. Microscopic examination of Gram's stain of 48 to 72 hour culture on lactose (Andrade) agar should show *no* spores. If spores were present, then the purification process outlined above was repeated, for it was observed that whenever this was the case, a *non-fermenting* aerobic spore former could be isolated while the pure fermenting type did not show spores on this medium in the designated time. The strains isolated resemble B. macerans described by Schardinger.

MORPHOLOGY

Vegetative cells. The organism varies in size on different culture media.

On nutrient agar, the vegetative cells appear (after 24 hours at 37°C.) as rods about as wide as B. coli, but 2 to 4 times as long. The size of the majority being 0.6 by 2.5 μ. They are grouped singly or in pairs; parallel forms were frequently observed and occasionally V forms were noted. The ends are rounded and the bacillus is slightly fusiform.

On lactose (Andrade) agar and in litmus milk the cells are somewhat longer, usually 3 to 4 μ. Spores were not seen.

In lactose (Andrade) broth they appeared especially elongated often measuring 6 and occasionally 8 μ. All of the 14 cultures showed an occasional spore on nutrient agar after 24 hours and after 48 hours at 37°, sporangia and spores were numerous. The endospores are elliptical, their diameter greater than that of the vegetative cells and located subterminally. The size of the majority of spores was 0.8 by 1.4 μ.

STAINING REACTIONS

After repeated observations and comparison with known cultures the organisms were considered to be Gram negative. This was particularly true if the cultures were grown on lactose media. The gentian violet stain is removed with difficulty and the Gram stain may easily be confused. The technique employed was to stain for 1½ minutes with aniline oil gentian violet then with Gram's iodine, to decolorize for 5 minutes with fresh 95 per cent alcohol and to counter-stain with dilute saffranin.

The Gram stain showed the vegetative cells to exhibit a tendency to granulation but in cultures from agar and Loeffler's blood serum

no granules were discernible when stained with carbol fuchsin, Loeffler's methylene blue or Albert's diphtheria stain. The vegetative cells stain readily with all of the stains mentioned.

MOTILITY

All of the 14 cultures were found to be motile when examined in a hanging drop from a 16 hour, 37°C. peptone water culture.

SPORE FORMATION

It has already been mentioned that spores were formed readily on nutrient agar in 2 days at the body temperature; it is significant to note, however, that spores could not be demonstrated in lactose agar or milk even after long incubation.

Three cultures were inoculated into lactose broth, lactose agar and plain agar and incubated at body temperature for 48 hours. No spores were visible in the lactose broth or lactose agar but they were numerous on plain agar.

On another occasion six cultures were inoculated into two batches of litmus milk. Microscopic examination (Gram stain) after 4 days and again after 10 days at 37°C. did not show any spores although the organism from these media was found to survive a temperature of 92°C. for 20 minutes. There was a marked tendency to granular staining.

Examination of four cultures on lactose (Andrade) agar, incubated 3 days at 37°C. then stored for 2 weeks in the ice box also failed to disclose any spores when stained by Gram's method. As in the milk cultures there was a marked tendency to granular staining and the cells were resistant to heat, surviving 92° for 20 minutes.

We feel therefore that the organism does not form recognizable spores readily, if at all, on lactose media. This is of some practical significance in water work as it indicates the improbability of detecting spores of this bacillus and thereby differentiating it from B. coli by examination of stained mounts from confirmatory lactose agar plates.

TEMPERATURE RELATIONSHIPS

Growth is much more luxuriant on agar at 37°C. than at 20° to 22°C. At the lower temperature 4 days may be necessary before any growth is visible. No growth was visible after one week at 53°C.

CULTURAL CHARACTERS

Plain agar. On this substrate the character of growth varies with the age and consistency of the medium. On moist, fresh agar, there is a moderate, spreading, effuse, glistening, transparent, butyrous growth which is difficult to see if the medium is not perfectly clear. On dry (high agar content) or old agar the growth is filiform and almost opaque and in old cultures (2 weeks) it becomes membranous. The medium is not changed, there is no distinctive odor and no chromogenesis.

Lactose agar. On fresh lactose (Andrade) agar, the surface growth is almost invisible on account of its effuse character and transparency. It spreads rapidly over the surface forming acid, and acid and gas in butt.

Gelatin. At 37°C. gelatin was not liquefied in 48 hours.

At 20° to 22°C. gelative stabs showed a moderate growth which was best at the top and filiform along the line of puncture. Liquefaction was very slow, first becoming evident in from 14 to 20 days.

Tubes evenly inoculated on the surface and kept for 30 days (20° to 22°C.) showed but 2 mm. of liquefaction.

Broth. In nutrient broth at 37°C. there was but slight clouding and very little sediment. Surface growth was not evident until the third day when a pellicle was present. In sugar broth (glucose, lactose, sucrose, maltose and inulin) there was no surface growth even on long incubation. (14 days)

Potato. The reaction on potato was particularly striking. In 24–48 hours at body temperature, the entire mass of culture medium was covered with gas and in 4 to 7 days, the potato was almost completely digested. The diastatic action was very marked with all of the 14 cultures studied. The organism evidently produces a powerful pectinase, as the medium is entirely disintegrated.

COLONY CHARACTERISTICS

Plain agar. On nutrient agar surface colonies when well isolated, were irregular in form with a lobate edge. They may be described as amoeboid. The colonies were smooth, glossy and effuse, showed no distinct internal structure and quickly confluesced. It is generally difficult to discern the colonies due to the transparency of the growth.

Subsurface colonies resembled those of B. coli, i.e., they were circular or elliptical with an entire edge and showed a granular internal structure.

Litmus lactose agar. The surface colonies in 24 hours at 37°C. were faintly acid, otherwise resembling those described for plain agar.

The subsurface colonies were slightly acid resembling B. coli.

Incubation for 48 hours increased the acid reaction.

Endo agar. The medium employed was the product supplied by the Digestive Ferments Company. On this medium B. coli was observed to give a distinct red colony but only a slight metallic sheen, if any. Inoculation was made only on the surface.

The organism under consideration showed faint, but hardly discernible, growth in 24 hours at 37°C. After 48 hours small flat, round and amoeboid colonies about 1 mm. in diameter, pink to red, with a more intensely colored edge and center, were developed. We would certainly not consider it colon-like but these colonies would have to be fished in compliance with the United States Treasury Department Standard.

Eosin methylene blue agar. Two media were employed; the simplified E. M. B. of Levine and the Difco product. The results were similar. There was no growth in 24 hours and after 48 hours small pinhead, discrete colonies about ½ mm. in diameter with a distinct metallic sheen were present.

GROWTH IN LITMUS MILK

All cultures were inoculated into two different batches of litmus milk, made from skim milk powder, and incubated at 37°C.

The litmus was rapidly decolorized (24 hours) and the medium acidified. After 4 days, coagulation could be induced by heating. (Control tubes of B. coli also failed to coagulate unless heated). There was no further apparent change until 24 to 30 days incubation when evolution of gas followed by coagulation and extrusion of a clear whey was noticed in 10 of the cultures. It was thought that this reaction might be due to some contaminating organism. but microscopic examination of all of the tubes of one set of milk cultures showed only Gram negative long rods (and with a single exception, no spores) resembling the organism under consideration

Four cultures were inoculated into milk, covered with melted paraffine and heated at 80°C. for 10 minutes (the so-called sporo-

genes test). The litmus was reduced, there was no coagulation of the medium and no apparent change in 10 days at 37°C. At this time the milk coagulated on heating, and melting the paraffine seal by gently heating showed that there was but a very small amount of gas developed in the medium. The organism does not give the characteristic B. sporogenes or B. Welchii test.

INDOL FORMATION

Tests for indol were made on all strains in 7 days culture of broth and peptone with negative results.

NITRATE REDUCTION

All cultures reduced nitrates to nitrites when grown in nitrate broth for 5 days at 37°C.

FERMENTATION OF CARBOHYDRATES

Glucose neutral red broth. Four cultures and B. coli as a control were inoculated into (*a*) freshly heated neutral red glucose broth and (b) old unheated neutral red glucose broth, in Smith tubes, and incubated at body temperature.

In the freshly heated medium B. coli formed 25 per cent gas in 24 hours, but there was no reduction of the neutral red, while in the closed arm of the tubes of the old unheated medium the indicator was reduced to a canary yellow as is to be expected for B. coli.

The test cultures reacted in a similar manner, i.e. the dye was reduced in the unheated medium but not in the same medium which had been freshly heated. The gas formation was very meagre, in no instance being more than 5 per cent. There was no change in 48 hours.

Glucose peptone phosphate. Seven strains and B. coli, as a control, were tested for acid and gas production in 0.5 per cent peptone, glucose, dipotassium phosphate in Smith fermentation tubes.

The results are indicated in table 2.

Starch peptone water. Five strains were grown in 1 per cent arrowroot starch peptone water (Andrade indicator). There was very vigorous fermentation with acid and gas production. Both hydrogen and carbon dioxide were formed and the Voges Proskauer reaction was negative.

Lactose broth. Growth in lactose broth (with Andrade's indicator) always observed in Durham fermentation tubes was not as vigorous as in the glucose phosphate or starch mediums above. After 24 hours there was usually a distinct acidity in the open arm while the inner tube often showed but little acidity, (usually in lower end) and a small amount of gas. After 48 hours the entire medium becomes distinctly acid and 5 to 30 per cent gas may be obtained.

Inulin, sucrose and *maltose broths* were all fermented with acid and gas production. In all cases acid is first formed in open arm. Gas formation was particularly vigorous with inulin. It seems to be a peculiarity of this organism that it attacks the complex carbohydrates much more vigorously than the hexoses.

TABLE 2

Growth in Clark and Lubs medium (48 hours, 37°)

ORGANISM	(ANDRADE)	GAS PER CENT	CO_2	H_2 FLAME TEST	V. P.
14 F1	+	20	+	+	−
14 F2	+	5	+	+	−
14 F1	+	40	+	+	−
14 FA	+	35	+	+	−
14 BC	+	30	+	+	−
12 - 1	+	45	+	+	−
8	+	25	+	−	−
B. coli	+	45	+	+	−

FERMENTATION OF CARBOHYDRATES IN SOLID MEDIA

Nutrient agar containing 1 per cent of the test carbohydrate and Andrade's indicator were employed. Incubation was at 37°C. for 10 days. It was observed that the more complex test materials, starch, inulin, dextrin and glycogen were particularly vigorously fermented in 2 days (as indicated by the extent of disintegration of the agar) and that after 4 to 10 days, the acidity usually disappeared and the medium reverted to a distinct alkaline reaction. Dulcitol alone was not decomposed.

Carbohydrates were tested with the results indicated in table 3.

VOGES PROSKAUER AND METHYL RED REACTION

Tests for acetyl methyl carbinol and acidity to methyl red were made on all strains in Clark and Lubs medium after incubation at body temperature for 4 days. The Voges Proskauer reaction was

negative in all cases. The reaction to methyl red was slightly alkaline or neutral. Growth was particularly vigorous in this medium and was accompanied by much foaming.

GROWTH IN SPECIAL MEDIA

Uric acid. There was no evidence of growth in Koser's uric acid medium after 4 days at 37°C.

TABLE 3

	14 UNKNOWN STRAINS			CONTROLS		(ACID AND GAS)
	Acid	Gas	Reversion	Para B.	B. coli	B. AEROGENES
Dextrose	+	+	Slow or negative	+	+	+
Levulose	+	+	Slow or negative	+	+	+
Galactose	+	++	Rapid, 4 day	+	+	+
Arabinose	+	+	Rapid, 4 day	+	+	
Mannose	+	+	Rapid, 4 day	+	+	
Xylose	+	++	Rapid, 4 day	+	+	+
Rhamnose	+	+	Rapid, 4 day	+	+	
Trehalose	+	+	Generally rapid	+	+	
Melezitose	+	+	Rapid, 4 day			
Lactose	+	+	4 to 10 day	−	+	+
Maltose	+	++	Rapid, 4 day	+	+	+
Sucrose	+	++	Rapid, 4 day	−	−	+
Mannitol	+	++	Rapid, 4 day	+	+	+
Glycerol	+	++	Rapid, 4 day	−	+	+
Dulcitol	−	−		+	−	−
Salicin	+	+	4 to 10 day	−	+	+
Dextrin	+	++	Rapid, 4 day	−	−	+
Inulin	+	++		−	−	−
Starch	+	++	Rapid, 4 day	−	−	+
Glycogen	+	++	Rapid, 4 day	−	−	−

Lactose bile. Six cultures were inoculated heavily into lactose bile (Difco). No gas was produced in 4 days.

Gentian violet lactose broth. In lactose broth containing gentian violet in a dilution of 1:100,000 there was no gas or other evidence of growth in 5 days at 37°C.

RESISTANCE TO HEAT

It has previously been mentioned that old cultures taken from lactose agar and milk survived a temperature of 92°C. for 20 minutes although no spores were visible on microscopic examination.

The following experiment was performed to check these observations: A loop of a 48 hour lactose (Andrade) broth culture was inoculated into each of 5 tubes of melted starch agar which were kept in a water bath at 89° to 91°C. After 10, 20, 30, 45 and 60 minutes, tubes were removed from the bath, cooled and incubated at 37°C. A Gram stain of the broth culture showed it to be a pure culture of long Gram negative rods and no spores were visible.

Plain and lactose agar culture suspended in broth were also heated as above. The results are given in table 4.

TABLE 4

Resistance of aerobic spore forming lactose fermenting bacilli to heat (89-91°C.)

TIME OF EXPOSURE	GROWTH IN STARCH AGAR (48 HOURS 37°C.)*		
	Inoculated from		
	Lactose agar	Lactose broth	Plain agar
minutes			
10	+	+	+
20	+	+	+
30	+	+	+
45	+	+	+
60	+	+	+
Microscopic examination of inoculum	Gram negative long rods. No spores	Gram negative long rods. No spores	Gram negative rods. Many spores

* Two cultures employed, 14B1 and 8.

IDENTITY OF ORGANISM

Meyer in 1919 described a sporing lactose fermenter which he isolated from Newport and Covington, Kentucky water. In many respects his organism is strikingly similar to the one here recorded (e.g., dulcitol is the only carbohydrate not attacked) and we thought that possibly they were the same, but rather important differences have been observed. The Meyer strain was non-motile, gave a positive Voges-Proskauer reaction, liquefied gelatin rapidly, and failed to reduce nitrates. The strain herein described is actively motile, negative for the Voges-Proskauer test, liquefies gelatine slowly and reduces nitrates very vigorously.

Two cultures have been described in connection with studies on acetone production which may be identical with the organism re-

ported in this paper. The B. macerans of Schardinger was isolated in 1904 from potatoes. From the meagre description available, it cannot be differentiated from the strain under discussion. In 1919, Northrop, Ashe and Senior described an acetone producing organism, also isolated from potatoes, which they named B. acetoethylicum. It is said to differ from B. macerans in that the latter does not ferment galactose and levulose with NH_3 salts as a source of Nitrogen.

We have not been able to obtain cultures of *B. macerans* or B. acetoethylicum for comparison with our strains. In the published reports the fermentation of lactose (with gas) by *B. acetoethylicum* is not recorded while *B. macerans* is said to form gas in milk (presumably from lactose). We will therefore consider our strains tentatively as B. macerans.

SANITARY SIGNIFICANCE

Little is known as to the sanitary significance of B. macerans and closely related forms. Such organisms have been isolated from potatoes, retting flax, white flour and water. There is no record that they are present in the intestinal tract although a careful search may disclose them. Information as to the distribution of these sporing, lactose-fermenting forms capable of growing aerobically is now being gathered and the pathogenicity of the isolated strains is also being investigated. The organism isolated by Meyer was non-pathogenic.

In a chlorinated water B. macerans would be present long after the ordinary water-borne pathogens had been destroyed. The detection of B. macerans, in the absence of organisms of the colon group, in a treated water should therefore not be considered an indication of danger from such intestinal disturbances as typhoid fever or dysentery. The presence of these sporing organisms in water interferes seriously with the routine tests for B. coli with which they may be confused and is possibly responsible for the poor results sometimes reported in water purification.

SUMMARY

A Gram negative sporing bacillus capable of fermenting lactose and growing aerobically was isolated from two chlorinated surface water supplies in Iowa.

The morphological, cultural and physiological characteristics are detailed.

The strain resembles markedly that described by Meyer, differing with respect to rate of liquefaction of gelatin, nitrate reduction and the Voges-Proskauer test.

The organism should be of particular interest to water works operators because of its extreme resistance to chlorination, and because of the ease of confusion with the colon group in routine tests as ordinarily performed. Its presence in water may explain anomalous positive colon tests. Information as to its source is particularly desirable.

The cultures isolated are strikingly similar to *B. macerans* and *B. acetoethylicum*.

NOTE ON PATHOGENICITY

The *Bacillus acetoethylicum* of Northrup, Ashe and Senior was reported non-pathogenic to mice.

One of the similar organisms isolated in the course of this study was tested for pathogenicity on rabbits in the following manner:

An agar culture was scraped to remove the entire growth on its surface and the material suspended in physiological salt solution and added to the drinking water supplied to a rabbit. This process was repeated daily for a week. The test animal did not show any untoward symptoms.

Another rabbit was injected intravenously with one cubic centimeter of a live culture prepared by removing the growth of organisms from an agar slant as indicated above and suspending the material in 10 cc. of sterile salt solution. The animal developed no symptoms that would indicate bacterial infection.

We are therefore of the opinion that this organism is not a pathogenic form.

REFERENCES

ELLMS, JOSEPH W. 1920 Report of experiments in the purification of the water supply of Milwaukee, Wisconsin.

EWING, C. L. 1919 Presence of a spore bearing aerobic gas forming bacillus in Baltimore city drinking water. Am. Jour. Pub. Health, ix, 157–158.

HALL, I. C., AND ELLEFSON, L. J. 1918 The elimination of spurious presumptive tests for B. coli in water by the use of gentian violet. Jour. Bact., iii, 329–354.

HALL, I. C., AND ELLEFSON, L. J. 1919 Further studies on gentian violet as a means of eliminating spurious presumptive tests for B. coli in water. Jour. Am. Water Works Assoc., vi, 67–77.

MEYER, E. M. 1918 An aerobic spore-forming bacillus giving gas in lactose broth, isolated in routine water examination. Jour. Bact., iii, 9–14.

NORTHROP, J. H., ASHE, L. H., AND SENIOR, J. K. 1919 Biochemistry of B. acetoethylicum with reference to the formation of acetone. Jour. Biol. Chem., xxxix, 131.

PERRY, M. C., AND MONTFORT, W. F. 1921 Some atypical colon aerogenes forms isolated from natural waters. Jour. Bact., vi, 53–69.

SCHARDINGER, F. 1906 Bacillus macerans, ein aceton bildender Rottebacillus. Centrbl. fur Bact. II, xiv, 773.

KOSER, S. A. 1918 The employment of uric acid synthetic medium for the differentiation of B. coli and B. aerogenes. Jour. Infect. Dis., xxiii, 377–379.

CLARK, W. M., AND LUBS, H. A. 1915 The differentiation of bacteria of the colon-aerogenes family by the use of indicators. Jour. Inf. Dis., xvii, 160–173.

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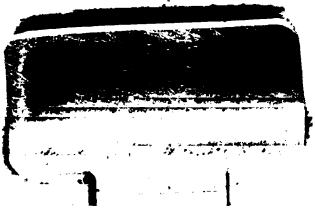
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